SOME EXPERIMENTS WITH AUXIN A AND OTHER GROWTH FACTORS.

by

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A Thesis submitted for the Degree of

MASTER OF ARTS

in the Department

of

BOTANY

The University of British Columbia

APRIL, 1938

ACKNOWLEDGMENT

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INTRODUCTION.

In the Fall and Spring of 1936 and 1937, auxin a, a plant hormone was isolated, and its identity confirmed. The Went test (72a) technique was developed to test its activity. That technique was experimented with in hope of finding some improvement on it. Other substances such as indole-3-acetic acid and γ-indole butyric acid were tested by the developed Went procedure. Pre-treatment of Avena seeds with hetero-auxin, urine, auxin a and ethylene-chlorhydrin vapour increased our knowledge and confirmed literature on seed pre-treatment with growth factors and dormancy breakers.

Whether or not auxin a played any role in animal tissue was accidently brought to our notice, and some interesting results were obtained which seem to conflict with opinion to date, that auxin plays no role in the animal kingdom.

Haning exhausted our supply of prepared auxin a, our attention was attracted by commercial preparations such as Hormodin-A, and a few evergreen and deciduous cuttings were treated in a hope of promoting rooting.

In the following thesis the author wishes to explain these experiments, their nature and results, and to conclude with a correlation of opinions to date on the nature and activity of auxin a and related substances.

In the early literature on the work in this field the non-committal terms, growth substance, Wuchsstoff, growth regulator and growth hormone were used, but as knowledge developed it became clear that substances causing cell elongation must be regarded as a separate group. Since recent work indicates that this group is heterogenous, the term "auxin", first suggested by Kögl and Haagen Smit in 1931 will be arbitrarily restricted to those substances which bring about the specific growth reaction which is conveniently measurable by the curvature of Avena coleoptile. In this thesis the terms phytohormone, growth substance (g. s.),growth hormone, growth factor and active factor will be used in the sense of auxins. The term Wuchsstaff in particular, has been used for some of the growth substances of lower plants, such as Bios. It cannot be too strongly emphasized that only those substances whose activity is determined on higher plants preferably by the standard methods described in Chapter 111 of "Phytohormones" by Went (1937), can be termed auxins. In this test strength of activity is measured in avena units (A. E.). By one A. E. is understood the amount of growth factor which causes in the test reaction of F. W. Went, a curvature of ten degrees in the Avena coleoptile. This test reaction will be dealt with later. The top of an oat sprout in one hour will produce, roughly, one A. E. A corn top, will in the same time, diffuse into agar, 1.3 A. E. (72b)

The realization that important functions in higher animals are controlled by chemical substances which act in extremely dilute concentrations has been shown with the progress in hormone and vitamin research. However, it is only in recent years that important functions of life among the higher plants have been shown to be similarly controlled. In fact, experimental proof of the influence of such substances in plant physiology was only achieved in the last two decades, although J. Sachs(57) in 1880 predicted the possibility of such inter-relations even before the hormone theory was introduced in animal physiology. To give an historical survey of the development of the hormone concept in plants,in an article of this type, would not give it the justice it deserves; moreover this information can be obtained in excellent detail in either " Growth Hormones In Plants" by Boysen Jensen (1936), or, "Phytohormones" by Went and Thimann (1937). The following chart might give the reader a brief idea of the stages of progress made since Darwin and Sachs work in 1880. (15)(57)

The unknown factor then appears in growing tips and may be diffused from there into agar blocks and tested on other decapitated Avena coleoptiles by the Went procedure.
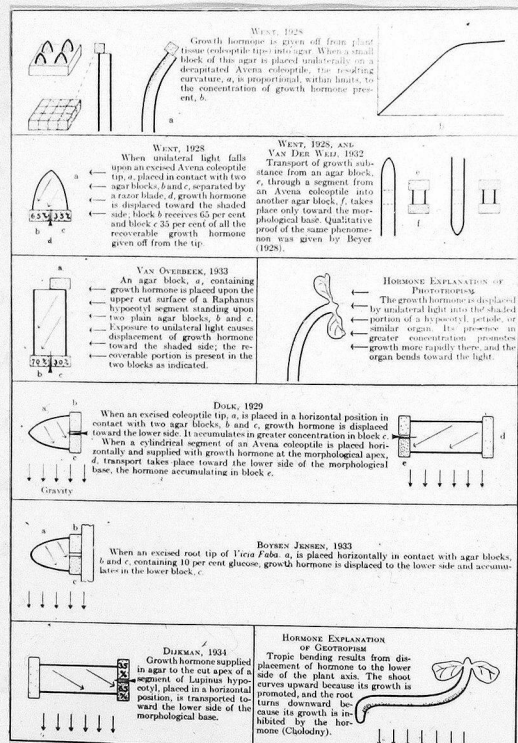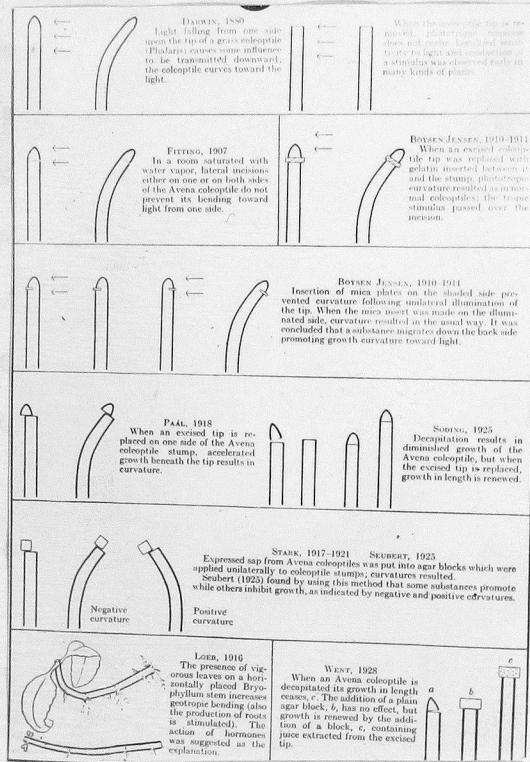
The Reason For Choosing Urine As A Source.

In the Fall of 1930 Kögl ät al, at the University of Utrecht, undertook the chemical investigation as to the nature of the growth hormone or hormones in plants, of which,at that time, very little was known. According to Went it was unlikely to be an inorganic compound and due to its heat resistance it was unquestionably not an enzyme. Obtaining it from Avena coleoptile tips and testing its coefficient of diffusion through agar blocks it was found to have a molecular weight of 376. Considering possible errors this would be somewhere between 300 and 400. [33a]

The success of a chemical investigation into a problem of this kinddepended on a specific test reaction, sufficient stability of the substance being examined and a reasonable supply of initial material. Previous historical work showed that the first two conditions at least, were fulfilled in regard to the plant growth factor. Their problem was to prepare the material and try to arrive at its chemical structure and physiological importance by a study of the actual crystalline hormone.

The first step was to find the most suitable material to isolate it from in sufficient quantities for testing purposes. Its isolation from grass tips, physiologically the most important source, proved unfeasible, as did many other sources which have proved themselves to have a factor with coleoptile bending ability. Experiments with a great variety of initial material were set aside however, when they found in human urine a source which proved by far the most satisfactory as to yield. For many years mammalian urine has been known to contain certain active factors in high concentration. It was probably with this hope

in view that pregnant urine which was known to contain follicle hormone
was looked to.

How great the difficulty of isolation from the
so far known plant material would have been could only have been app-
reciated after they realized the activity of the crystalline substance.
Calculating a yield of 10% pure substance they would have needed enough
raw material for chemical study to net at least one gram of the factor.
This would call for ten billion decapitated corn plants or cultures of
Rhizopus reflexus amohnting to thirty-nine hundred meters giving nine-
thousand, four-hundred litres of culture fluid. With E.coli the sur-
face question would be eliminated but twenty-five thousand litres of
culture fliud would be needed; similarly the waste water of thirty
thousand Kgs. of baker's yeast or the heat extraction of two thousand Kgs.,
or plasmolysis of one thousand Kgs. of it, is out of the question. On
the other hand human urine contains one gram in five hundred litres(33b).

Their choice should be considered fortunate, in
as much, as the coleoptile bending effect in extracts of these lower mem-
bers of the plant kingdom is probably due to indole-3-acetic acid, a
break-down product of tryptophane and not due to the more powerful and
complex factor to be found in human urine.

Another factor to consider before they could
judge the suitability of the initial material was to draw certain conclusions
from the enrichment which would have to be in each particular case before
getting at the pure substance. For example, the active substance from the
culture fluid of Rhizopus reflexus could only be obtained pure after four-
hundred and fifty thousand to one-million, two-hundred and fifty thousand
times concentration. With human urine only twenty-one thousand times
concentration is necessary. As a comparison, may be mentioned here, that

in the purification of the follicle hormone from pregnant urine as originally used by Kögl, a fifty-four thousand times concentration was necessary.

Deciding on urine as their source of their hormone the next step was to decide if human, animal, pregnant or mixed was the best source. This question probably arose in their mind due to dietary and other differences. To test these sources for activity they adopted, as in previous work, the Avena unit (A. E.). The results of these tests were as follows: (33)

Human Urine	A. E. per mg.
Average of 140 cases	2400
Normal variation	1000 to 5000.
Pregnant urine(average of 14 cases)	1350
Extreme values	250 to 13,000.
Horse Urine	
Stud	2000
Mare (5 samples)	1800 to 7000
Cow Urine	
Two samples	65 to 240
Pig Urine	
Four samples	50 to 180

It was to their advantage in their original work, to use urine from pregnant humans, in as much as commercial follicle hormone could be obtained as a by-product.

This preceding review has then, it is hoped, served in some measure to explain why we chose human urine as our source.

Extraction From Urine.

Extraction procedures were taken in a large measure after Kögl and Erxleben(5a).Due to the distillation facilities and time factor at our disposal, a total of fifteen litres of relatively fresh male urine with an expected factor content of about thirty mg . was

mixed with HCl (1.1N) until distinctly acid to Congo Red, and distilled down to a rich syrup by the batch process. For example: November 17th. at 5 P. M. in a two litre Claisson flask were distilled 900 ccs. of urine down to two-thirds volume. At 8 P. M. another 325 ccs. were added and the whole brought down to a syrup and placed in refrigerator. November 19th. at 7:30 P. M., distilled down, and at 9:45 P. M., added another 350 ccs.

EXTRACTION PROCEDURES

PROCEDURE 1. Evaporation of Urine.

Nov. 17--Distilled 1225 ccs. to rich syrup and put in refrigerator.

Nov. 19--Added   1350 ccs. and distilled down and put in refrigerator.

Nov. 21--Added   1625 ccs. and distilled down and put in refrigerator.

Nov. 26--Added   2250 ccs. and distilled down and put in refrigerator.

Nov. 28--Added   500 ccs. and distilled down and put in refrigerator.

Dec. 1--Added   2350 ccs. and distilled down and put in refrigerator.

Dec. 4--Added   2380 ccs. and distilled down and put in refrigerator.

Dec. 6--Added   2320 ccs. and distilled down and put in refrigerator.

Dec. 9--Added   1000 ccs. and brought total down to rich syrup. The residue was dark brown with some needle-like crystals and contained the expected growth factor.

Procedure 1 was preceded by considerable trial and error before a satisfactory way of reducing frothing and handling apparatus could be determined. Degree of vacuum and temperature control appeared to be the two limiting factors in this respect and constant watch, accompanied by slow distillation were necessary throughout. If apparatus is set up as in underlying diagram it will be found to be as as satisfactory as possible.
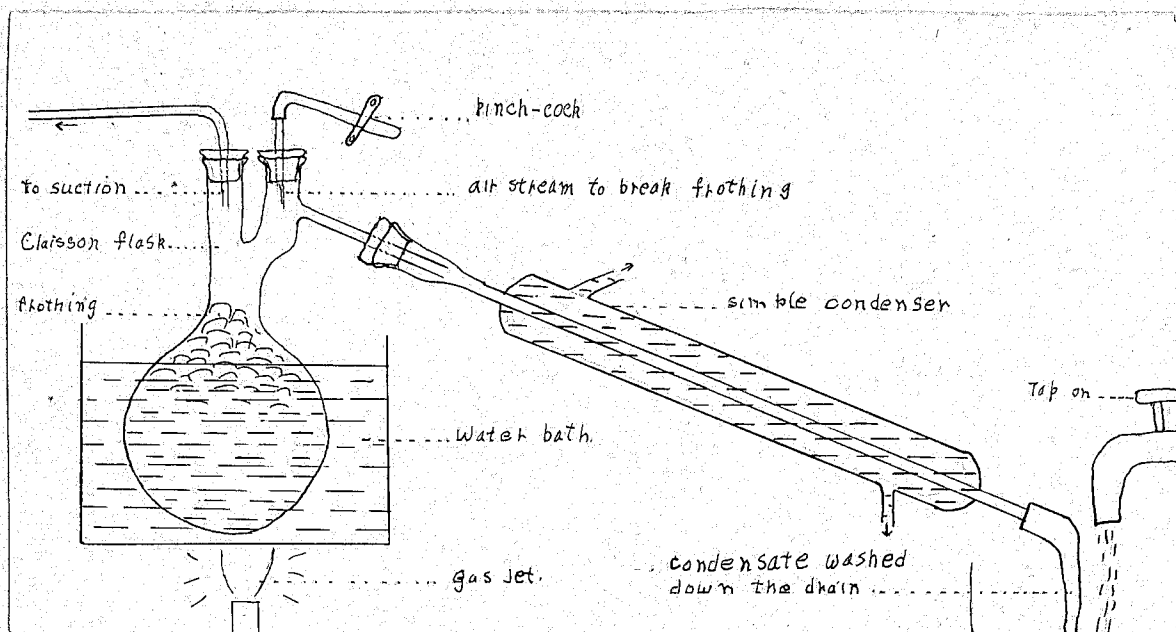
Figure 2.

Procedure 2. Ether Extraction of the raw syrup.

The syrup was then diluted to 3 litres with
distilled water, again distinctly acidified with HCl (1.1 N) and extracted
4 times with equal volumes of peroxide free ether in a 500 cc. heavy
separatory funnel.

Procedure 3. Drying of Ether Extract.

The collected ether extracts now containing the
active factor were dried over anhydrous sodium sulphate by adding the
salt to large jars containing the extract and letting stand overnight.

Procedure 4. Concentration of ether extract.

The ether extracts were now evaporated to as near
dryness as possible. This was of course a batch process again adding
periodically fresh volumes of ether to the accumulating concentrates.
The brown, oily residue left contains almost the total of growth factors
in an enrichment of approximatly sixty-five times. It was found that
great care must be exercised at this point in the respect of good ven-
tillation and warm water-bath temperatures only. Hot plate must be

removed and flask cooled by immersing base in cold water before removing
cork to supplement the depleting extract. In the event that this proced-
ure is not followed, when the cork is removed the fumes from the hot
liquid may rise out and flow down over the hot plate and cause combustion.
Ordinarily it should be unnecessary to mention such details, but we find
in this case caution can not be over-emphasized.

<u>Procedure 5</u>. Fractionation with bicarbonate solution.

The residue of P. 4 was dissolved in 150 ccs. of
pure ether and eight times shaken with sodium bicarbonate solution,
using 50 ccs. at a time. The bicarbonate extracts are poured together
and acidified with HCl (1.1 N) until distinctly acid to Congo Red and
eight times extracted with ether, using 50 ccs. of ether at a time. It
is interesting to note that the colour disappeared when the auxin was
a sodium salt and returned to the original orange colour when acidified
again. The ether extract was dried with sodium sulphate as before and
evaporated using only hot water from the tap as a source of heat, except
towards the end. The residue weighed approximately 4.5 grams, enrich-
ment 78 times.

<u>Procedure 6</u>. Extraction with petrol ether.

The ether residue from Procedure 5 was next put
on a water bath with 40 ccs. of petrol ether (Caution B.P. 40-60° C.) and
refluxed under double condensors for $\frac{1}{2}$ hour. After cooling the petrol
ether was decanted and the process repeated twice more with fresh petrol
ether. The ineffective impurities dissolve in the petrol ether.

<u>Procedure 7</u>. Extraction with ligroin.

To the residue in Procedure 6 from which the
petrol ether had been only decanted, we now added 40 ccs. of ligroin
(B.P. 100-120° C.) and for $\frac{1}{2}$ hour heated it to 90° C, decanted and this

process also repeated twice and then freed of ligroin by distillation in vacuo. The ligroin also dissolved additional ineffectual impurities. The residue was now about 2 grams and the concentration 238 times.

Procedure 8.   Extraction with Benzol

The residue from Procedure 7 was dissolved in 30 ccs. of 60% ethyl alcohol and the solution extracted by shaking ten times with Benzol, using 10 ccs. at a time. The total 100 ccs. of Benzol solution was now itself extracted 3 times with equal volumes of water and then again 3 times with an equal volume of 50% methyl alcohol.

The methyl alcohol extracts were evaporated to dryness, the residue was united with the water extracts and the whole four times extracted with ether by shaking. The ether extract was then evaporated, leaving about $\frac{1}{2}$ gram of residue at an enrichment of 600 times.

Procedure 9.   Lead Salt Precipitation of Impurities

The ether residue of Procedure 8 is dissolved in 15 ccs. of 96% alcohol. To this was added a concentrated aqueous solution containing .5 grams of neutral lead acetate. The precipitated impurities were then filtered off and washed with alcohol. To the active filtrate was added 30% NAOH drop by drop till weakly alkaline. This brought down a precipitate amongst which was the sodium salt of the hormone. Kogl did not have good success at precipitating all his active factor in this way, so rather than dissipate our factor among several fractions it was decided after consultation to convert the hormone back to its original acid pH. by adding glacial acetic acid, this dissolved the precipitate. This solution was extracted 4 times with equal volumes of ether and evaporated to residue. Enrichment is now in the neighborhood of 1000 times, and the residue about .3 grams.

Procedure 10. Calcium Salt Precipitation.

The residue of Procedure 9 was dissolved in 3 ccs. of alcohol and diluted with 30 ccs. of water. Next a concentrated aqueous solution of 16 grams of calcium acetate was added, and then under repeated shaking with normal KOH until no more precipitate comes down upon adding further drops. The precipitate was then filtered off and washed repeatedly with aqueous alcohol (10%). It contains then only traces of the active substance probably adsorbed on to the crystals. The filtrate was then acidified with glacial acetic and extracted with ether 4 times. The ether residue after evaporating to dryness represented about .2 grams at an enrichment of about 1600 times.

Due to our limited vacuum distillation equipment it seemed rather pointless to proceed with Kogl's next step of esterification and lactonization. Inasmuch as auxin a lactone is no way near as active as auxin a; and although it might be hydrolized back to auxin a it is possible our factor might have been dissipated in fractions.(7a) Moreover the point of esterification, as the author sees it, is to make a lower B.P. product and thus facilitate vacuum distillation, fractionating a procedure which we were unwilling to embark on as we hesitated to risk the loss of the active factor, due to our limited equipment for distilling under high vacuum. In addition we were interested in obtaining a highly active concentrate with which to do some experiments and not a crystalline product for structural analysis. For this reason then the author did not consider the dissipation of his product through esterification, respective lactonization and high vacuum distillation and its temperature fractionations would be justified.

Procedure 11.

Subjected residue of Procedure 10 to as high vacuum as possible, (.01 mm.) and 100^b C. According to Kögl the first fore-run of auxin would distil over at .005 mm. and 125^b C. Therefore this was as far as we carried our procedure.

The syrup was taken up in 5 ccs. of a dilution fluid and bottled (containing 160 mg. of Kcl and 0.2 ccm. of acetic acid/L.) In physiological work the transport of growth-factor is probably handi-capped in the absence of such a solution.

Procedure in Brief for Preparation of Auxin a Concentrate.

Retained   Discarded

Urine 15 Litres
Acidify and distil-------------------------------------->Urine distillate

Urine syrup
Dissolve in water, acidified and extracted with ether)
              -------------------------------->Water residue

Ether Extract
Dry over Na$_2$So$_4$ and evaporate syrup
              ------------------------------> Ether distillate

Ether Residue
Dissolve in 200 ccs. ether and extract eight times
with 50 ccs. of saturated Na. HCO$_3$
              ------------------------------> Ether Layer

Bicarbonate Fraction
Acidify, extract eight times with ether, dry over
Na$_2$So$_4$ and evaporate to syrup.
              ------------------------------> Bicarbonate water
                                                 solution

Ether Residue
Reflux 3 times with 40 ccs. of petrol ether each
for $\frac{1}{2}$ hour and ether decanted off.
              ------------------------------> Petrol Ether

Petrol Ether Residue
Reflux 3 times with 40 ccs. of ligroin each for
$\frac{1}{2}$ hour and decant, then high vacuum heating to
drive off ligroin.

---------------------------------------> Ligroin solution

↓

Ligroin Residue
Dissolve in 30 ccs. of 60% ethyl alcohol and
extract 10 times with 10 ccs. of Benzol by
shaking.

---------------------------------------> Alcoholic solution

↓

Benzol Extract
Extract 3 times with equal volumes of water
and then extract 3 times with equal volumes
of 50% methyl alcohol.

---------------------------------------> Benzol Layer

↓

Methyl and Water Extracts
Evaporate alcohol extract to dryness, add
water extract and extract 4 times with ether.
Evaporate to dryness

---------------------------------------> Water layer

↓

Ether Residue
Dissolve in 15 ccs. alcohol and precipitate
impurities with lead acetate (.5 g.)
concentrated and neutral

---------------------------------------> Lead precipitate

↓

Filtrate from Lead Salt Precipitation
Acidify with glacial acetic and extract 4
times with ether and evaporate to syrup.

---------------------------------------> Water layer

↓

Ether Residue
Dissolve in 3 ccs. alcohol, dilute with 30
ccs. water and precipitate impurities with
.6 g. of calcium acetate in concentrated
aqueous solution. Add normal KOH until no
more precipitates. Wash precipitate with aqueous
alcohol.

---------------------------------------> Calcium precipitate

↓

Filtrate from Calcium Precipitation
Acidify with Glacial acetic and extract 4
times with ether.

---------------------------------------> Water layer

↓

Ether Extract
Distilled to heavy viscous syrup under high
vacuum and boiling water bath for five hours.
Take up residue in physiological dilution fluid
and little alcohol.

---------------------------------------> Ether distillate

Developing of Went Test Technique and Testing of Auxin a syrup.

It now remained to test the auxin a for activity. This necessitated first making the apparatus and then developing and practicing the technique using B-indole-acetic acid in the primary tests, a fairly cheap commercial preparation known to have a similar test reaction as auxin a but to a lesser degree. It seems rather premature to plunge into this phase of the work without first explaining the physiological effect of auxin a on Avena coleoptiles, which has so fortunately led to such a manner of testing its presence either qualitatively or quantitatively.

In demonstrating the presence of growth substances the coleoptile of the Avena seedling has been used almost exclusively as a test object. Its structure and sensitiveness to stimuli make it suitable for quantitative tests as well as qualitative demonstrations. For this reason practically all the facts known about plant hormones and most of the theoretical conclusions are based upon the Avena test, which in itself again is based upon growth in length of the Avena coleoptile. A minute amount of growth hormone applied unilaterally to the decapitated tip brings about increased cell elongation on the side receiving the growth substance, and this produces a growth curvature. The amount of curvature can be used within certain limits to indicate the concentration of the applied growth substance.

Before describing the test the structure of the young oat plant will be explained.

The Avena Seedling

Upon germination of the seed the primary root begins to grow out and is followed within one day by two secondary lateral roots. Meanwhile the shoot also starts to elongate. It consists of the

growing point, a very short stem and two partially developed leaves and a surrounding sheath, the coleoptile.
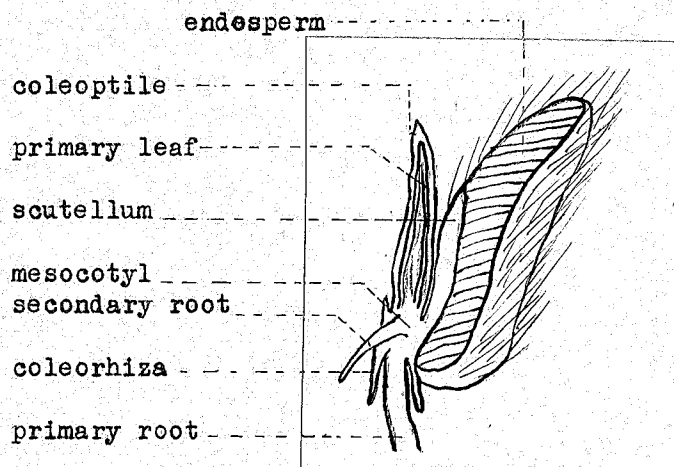
Figure 3. (72 zf)

   Between the coleoptilar node and the insertion of the scutillum there develops an internode generally called the mesocotyl, or in older literature the hypocotyl. The length of this mesocotyl varies on the treatment of the seedling. If the coleoptile be considered one cotyledon and the scutellum the other, then this internode is truly the mesocotyl (ie. between cotyledons). On the other hand if the coleoptile be considered the first ~~time~~ true leaf then this mesocotyl is simply the first internode or (epicotyl). Since the term mesocotyl has been generally adopted in physiological literature we shall retain its use.

   The growth of the coleoptile which is a hollow cylinder with a solid conical top places takes almost entirely in the longitudinal dimension. In its early growth up to a length of 1 cm., cell divisions of the parenchyma accompany the elongation (Avery & Burkolder, 1936) (1). The epidermal cells however cease dividing at a very early stage and grow only by extension. From a length of 1 cm. up to its final length (5 - 7 cm.) cell divisions are practically absent (Tetley & Priestly, 1927) (66) and growth is entirely by cell elongation at this

stage. On this account the coleoptile is a suitable object for studies of growth not complicated by cell division and whatever conclusions are drawn from it apply only to cell elongation. It is believed this cell elongation to be brought about in some way by a phytohormone produced in the tip. (72c)

In transverse section, the coleoptile is elliptical, with the short axis in the plane of symmetry of the seedling. One small vascular bundle runs up on either side (Fig. 4). The cells at the tip of the caleoptile are morphologically distinguishable from the others by the fact that they do not elongate and are almost isodiametric (Fig. 5). The epidermal cells of the extreme tip stain somewhat more deeply than the rest and are presumably richer in protoplasm. The region of iso-diametric cells is limited to the uppermost 0.5 mm. of the length of the coleoptile (du Buy & Nuernbergk, 1932) (8).

Cross section 5 mm. from tip
dorsal side faces seed.

pore

Tip

middle

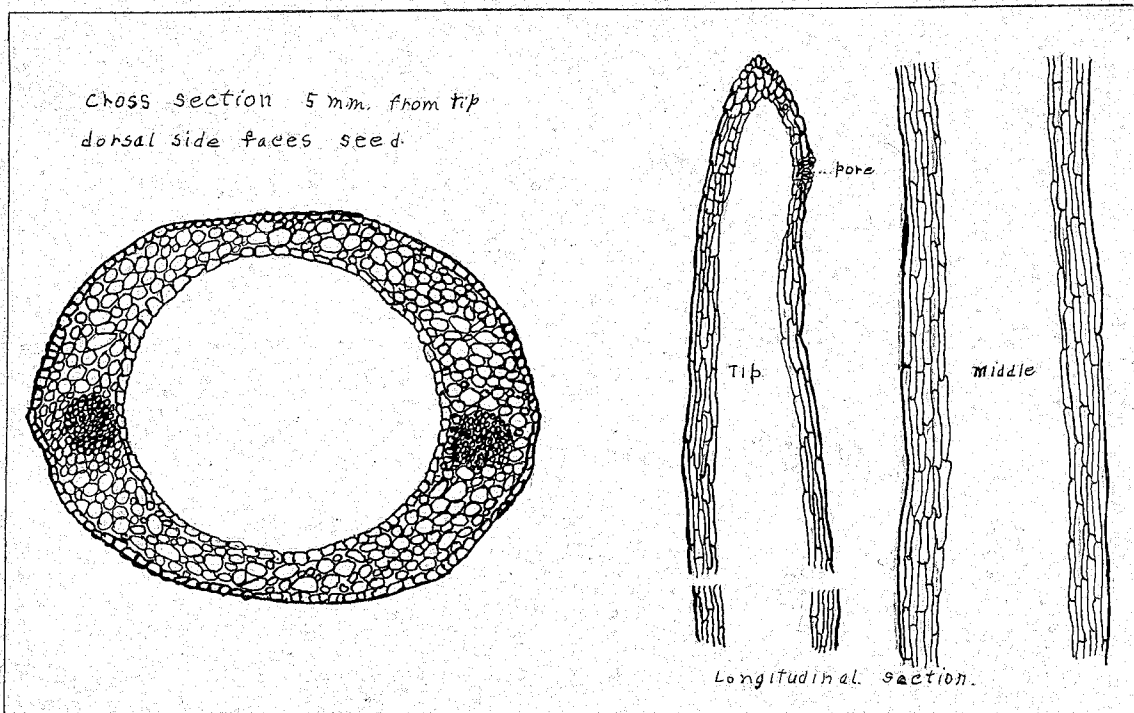Longitudinal section.

fig.4.   fig.5.

The growth of the primary leaf closely follows that of the coleoptile so that under normal conditions that coleoptile is almost completely filled. At a length of 50 - 60 mm. the coleoptile is broken through, near the tip, by this leaf, which then starts to grow very rapidly. At this time the growth of the coleoptile ceases. The whole period of growth lasts about 100 hours at 25° C. and the maximum growth rate which is reached at an age of about 70 hours is approximately 1 mm. per hour. (F. W. Went, 1937) (72d). For experimental purposes, coleoptiles 25 - 35 mm. in length, growing at a maximum rate are used.

During the growth of the coleoptile from about 20 mm. on, the upper most 2mm. scarcely elongate at all. The zone of maximum growth is first located near the base and migrates upwards and finally remains about 10mm. below the tip. At about 40 mm. length the growth of the most basal zones ceases altogether. (F. W. Went, 1937) (72d).

Preparation of Test Equipment.

The test objects have to be manipulated under orange or red light because they are exceedingly sensitive to the shorter wave lengths (under 550mµ) (Silberschmidt, 1928) (61). These shorter wave lengths if unilatterally illuminating the coleoptile cause phototropic bending while, if symmetrically distributed they cause a decrease in sensitivity to the applied auxin. According to Went, Corning light filters 243 or 348, or Schott's O.G. 2, are very suitable to cut out the phototropically active wave-lengths from incandescent lamps. With this point in mind, all experiments on test objects were carried out in the photographic dark room.

A relative humidity of about 90% and a temperature of 22° - 23° C. was also considered advisable; to secure this condition (72e),

seedlings were grown before and during testing in a light-proof glass case. This case was about two feet in length with a removable vaseline-sealed top and wet towel-paper lined interior. By its use a convenient temperature and humidity was easily maintained.

The next step was to devise some kind of holder for the oat sprouts. A three inch quarter inch glass tubing was cut, and to its end were sealed two smaller lengths after the fashion shown in Figure 6. The red hot glass is very conveniently handled, enlarged or pulled out into a lip with the aid of a cold file and tweezers. As soon as the tools heated up they would stick to the glass. Some four dozen of these were made of uniform size and shape. These were ajustably held in saw slits in a 1" by 1" by 18" bar of wood by means of small sheets of copper stripping. One rack contained a dozen or more holders and the box would hold as many as three racks at a time.

To supply the seedlings with the necessary water galvanized trays were contructed 1" by 1" by 15" in length. These were lined with paraffin wax to eliminate any toxic effect from the zinc. It was also found that lining the coleoptile holders and seed seats with wax not only prevented wetting by the water but improved the hold on the object. A holder in rack, coleoptile and tray, appeared very much as in Figure 7.
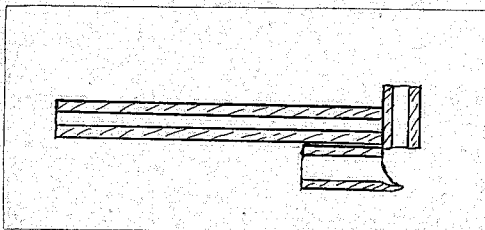
Figure 6.
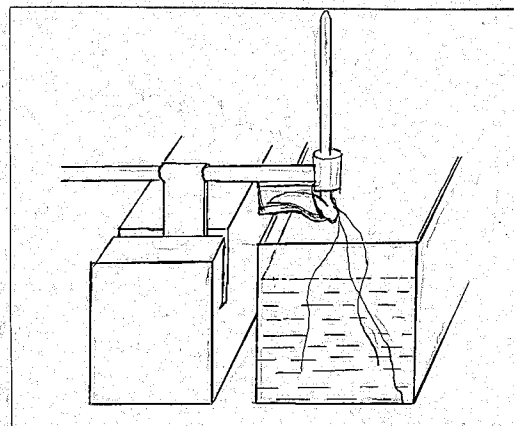
Figure 7.

Preparation of Test Objects

Inasmuch as most experiments to date in this field have been carried out with the genetically pure line of oats known as Victory Oats it was considered advisable to use the same strain. These were obtained from the experimental farms at this University. (7C)

Pre-soaked - 2 hrs.   Sowed in Petri dishes
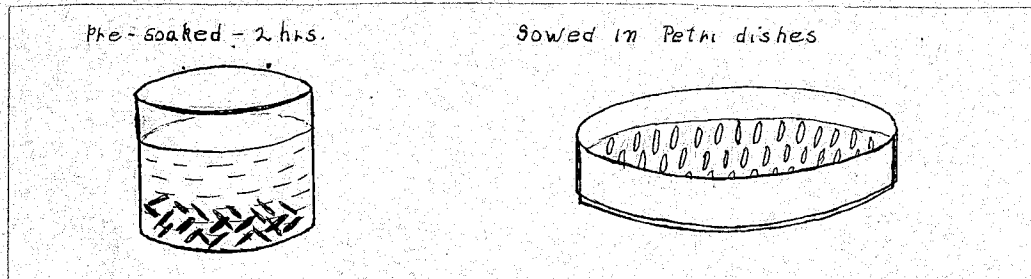
Figure 8.

The procedure was as follows: The seeds were freed from their husks (or glumes) and soaked in water for 2 hours. fig. 8. After this they were placed groove downwards on wet towel paper in Petri dishes and put in the dark room. Wet paper was on the inside face of the lid. After about 20 hours they were exposed to red light to suppress subsequent mesocotyl growth which would affect their sensitivity as test objects. Subjection to red light for 1 hour was followed by 10 hours more in darkness. The germinated seeds were then inserted in the previously prepared little glass holders, in such a way that the shoot comes under the centre of the guide and the roots point downwards. The clips were free to move in a vertical plane while the holders could be rotated and thus any adjustment of the growing plant was possible. They were then placed in the case to grow. About 48 hours after planting, the rack of caleoptiles now 20 - 30 mm. long were lifted out and ready for decapitation. It was found advisable to bring all shoots in a strictly vertical position at least 2 hours before use. When grown to the proper size they were selected for straightness and uniformity, and since the holders

were removable 12 - 15 good plants could be assembled together on the
same rack.

The first surgical step in the preparation of the
test plants was to cut off the extreme tip with a razor and then to place
the rack of objects back in the case. (See steps in accompanying figure 9).
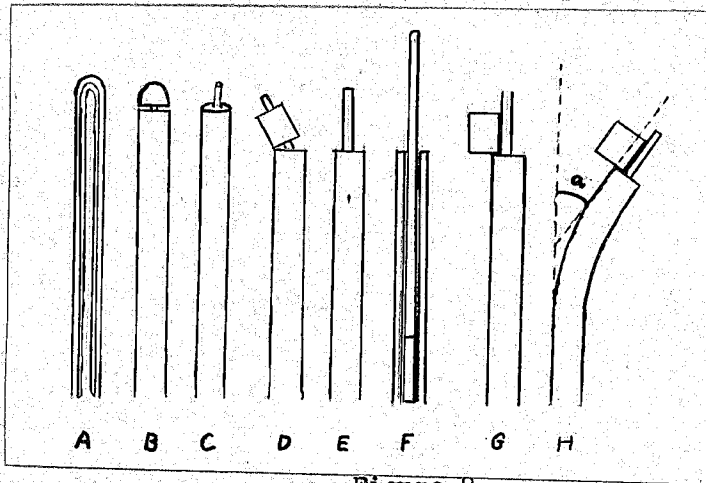
Figure 9.

In the subsequent period a large amount of growth
hormone present in the stump is being used for its residual growth and the
test plants become more and more sensitive to any hormone which is applied
(Van der Weij, 1931) (70). After three hours the topmost 4 mm. of the
stump is cut off. This was done by making an incision on one side of the
coleoptile without cutting the primary leaf; the top of the coleoptile
is then bent so that it breaks at the incision and the topmost part is
pulled off. The primary leaf which now protrudes 5 mm. or more is then
pulled gently so that it breaks at its base and is partially drawn out
(See Fig. 10). Any water congesting on the cut surface was blotted off
with a piece of dry filter paper. The small block of agar to be tested
is then placed against the drawn leaf and pressed down directly over
one of the two vascular bundles. It seems to be held in place by capillary
attraction. It represents about $\frac{1}{2}$ hours operation to decapitate and apply

the agar blocks to 12 coleoptiles. The rack of test objects are now placed back in the glass case, with the roots hanging in water and the lid of the box sealed by vaseline. This maintains a suitable temperature and humidity. Maximum results are abtained with a relative humidity of 90% and a temperature of $22^o - 25^o$ C. (33a)

After three hours the rack is removed and the bending of the coleoptiles is recorded by placing a strip of phototropically active printing paper closely behind the plants. They were then illuminated from in front by a projector which eliminated penumbra.

The degree to which the coleoptile had curved from the vertical could be measured with a protractor. The angle measured was that between the tangent to the extreme curved tip and the straight base. (See Fig. 9) The curvature over a range of 1° to 20° are strictly proportional to the concentration of g.s. in the agar. (10° represents 1 A.E.) If the curvature is much greater than 20 degrees ("Maximum Angle") (22f), the direct relationship between curvature and concentration no longer exists. Degree of curvature and a consideration of the dilution factor were used to calculate the strength of our solutions.
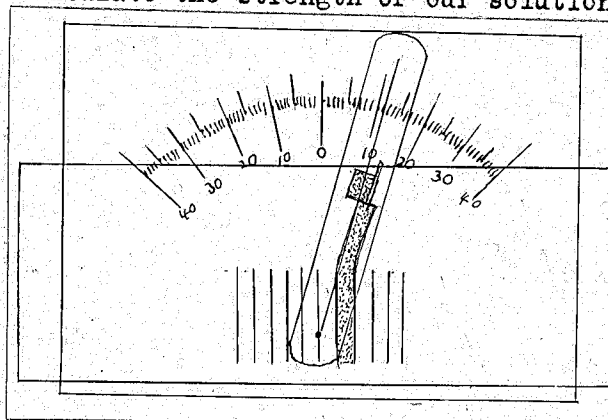
Figure 10.

The red light under which our operations were conducted was tested for its phototropic activity on the coleoptiles and

it was believed to be phototropically inactive within the limits of error. Much time was lost in trial and error early in the work and it was not until many racks of coleoptiles had been grown that there were produced a dozen properly decapitated test objects from several dozen seedlings. Control of manipulation technique is difficult but essential.

Preparation of Test Solution and Agar Blocks.

As previously stated the Went test technique was first practiced with B-indole acetic acid. 10 mg. of crystalline heteroauxin were weighed out and dissolved in 10 ccs. of Kogl's dilution fluid. This gave a solution of approximately .01 in 10 equals .001 concentration. (Kogl's Dilution fluid contains 160 mg. of KCl and 0.2 ccm. of acetic acid per liter of distilled water). Taking 1 cc. of this and adding it to 9 ccs. of dilution fluid a solution of .0001 original concentration was obtained. Similarly taking a cc. of this last solution and adding it to 10 ccs. of fluid dilutions of .00001, .000001, and .0000001, respectively were obtained. This tested to a Ph. of 4.8 as was desired by Kogl (33e). Solutions were kept in the dark to prevent precipitation.(47)

A 3% solution of 24 hour washed agar was now prepared and cut into small blocks which were dropped into the Erlenmyers containing the solution to be tested. It was assumed that the concentration of the B-indole-acetic acid in the agar block attained that of the solution in which they were soaking.

When the coleoptiles were in a decapitated condition and ready, a block was removed from the solution to be tested, cut to the proper size, that is (2 mm. by 1mm. by 1 mm.) and placed in position (Fig. 9). According to Van der Weij (70) small deviations in

size of the block has very little influence on the amount of curvature,
since this is dependent on the growth substance concentration and not
upon the amount of growth substance. However the amount of contact sur-
face between the agar and the coleoptile should always be the same. It
was found the degree of curvature was always much greater when the block
is placed over a bundle than when placed on parenchymatous tissue
(Laiback & Kornmann, 1933 b.) (46). Hence blocks had to be placed in
contact with vascular bundles if consistent results were to be obtained.
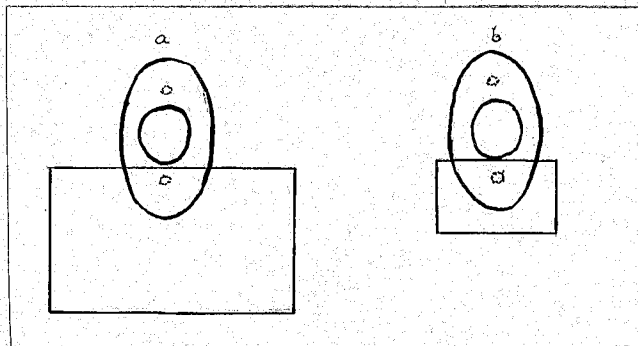
Figure 11.

The contact area is the same in both a and b though the volume of one
block is 8 times that of the other. (Thimann & Bonner, 1932) (69).

Preliminary work with Heterauxin.

Testing Heterauxin then (ie. B-indole-acetic)
we found it to be active at dilutions as low as .000001 and it might be
well to add at this point that an image of the coleoptile was drawn as
it stood before application of the agar block and again after time had
been allowed for bending. This gave something to compare its final
position with, and although relatively perfect specimens as regards
uniformity in size and straightness were used in later auxin a work, at
this practice stage we wasted as few as possible. Although excellent
results were obtained with Heterauxin in dilutions of .0001, .00001,
.000001, .0000001, it can not be said they were any better and certainly

not less interesting than the results with the extraction prepared in this laboratory. Having developed the technique of growing, handling and evaluating the test objects the time appeared opportune to test the extract, which there was reason to believe had by now lost its activity due to self inactivation. (see discussion later)

Testing the Auxin a.

Taking 1cc. of our auxin a concentrate in a pipette it was added to 9 ccs. of Kogl's dilution fluid. After mixing well, 1 cc. of the .1 concentrate was added to 9 ccs. of dilution fluid. In this way solutions of 1, .1, .01, .001, .0001, and .00001 of the primary dilution (5cc) were made up. Into this solution the small blocks of agar were dropped at least 2 hours before testing . (72g) With test solution ready the prepared coleoptiles were tested to find out roughly at what dilution our concentrate was active. A time outline of the procedure followed might be enlightning at this point.

1. Monday   8:00 A.M.   Husked oats and soaked in water until 10:00 A.M.

2.   10:00 A.M.   Placed germinating oats on moist towel paper in petri dishes.

3. Wednesday 8:00 A.M.   Transferred germinating oats to glass holders.

4. Thursday 8:00 A.M.   Put agar blocks to soak in dilutions to be tested.

5.   11:00 A.M.   Oats ready for use about 25 to 40 mm. long. Cut off extreme tip with a razor and placed the rack in case.

6.   2:00 P.M.   Topmost 4 mm. of stump is removed and enclosed leaf pulled loose and partially drawn out, the topmost end clipped off and the agar block cut and set in position contacting a vascular bundle.

7.   2:30 P.M.   The racks of completely set test objects were placed in the case.

8.   5:30 P.M.   The racks are removed from the case, the angles of curvature measured and shadow graphs made on printing paper.

7:00 P.M. The shadow-graphs can go in the wash water, complete
the record. At least 8 hours are needed to do the
actual testing once the objects are ready.

Following this procedure the activity of the auxin
a concentrate was shown to be present in toxic amounts in one and .1
concentrations of the original dilution of 5 ccm., and to cause curvature
at .01 with activity disappearing at .001 concentration. At .0001 and
.00001 visible activity had disappeared. Controls of Kogl's dilution
fluid and straight water-soaked 3% agar showed no bending. A summary of
these results with some of the shadow-graphs obtained during the work are
found below.

Figure 12.

Concentration.

1.0 ....

.1 ....

.01 ....

.001...............................
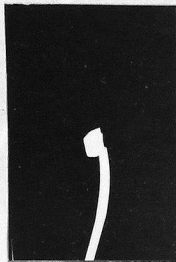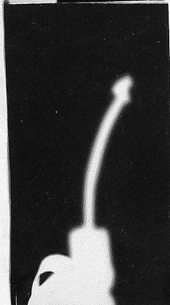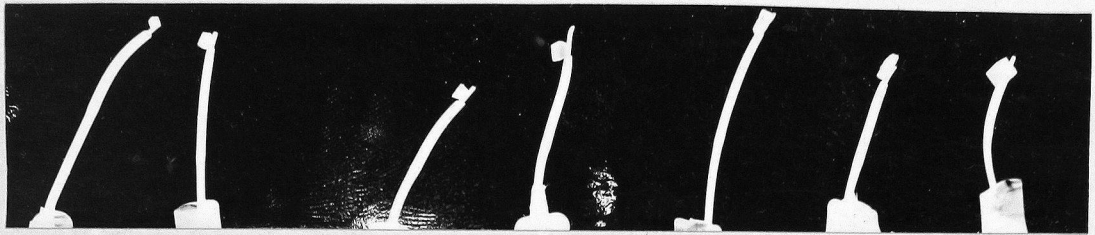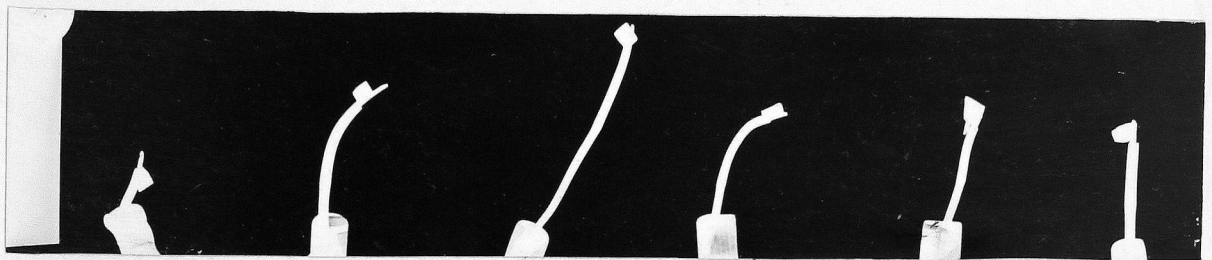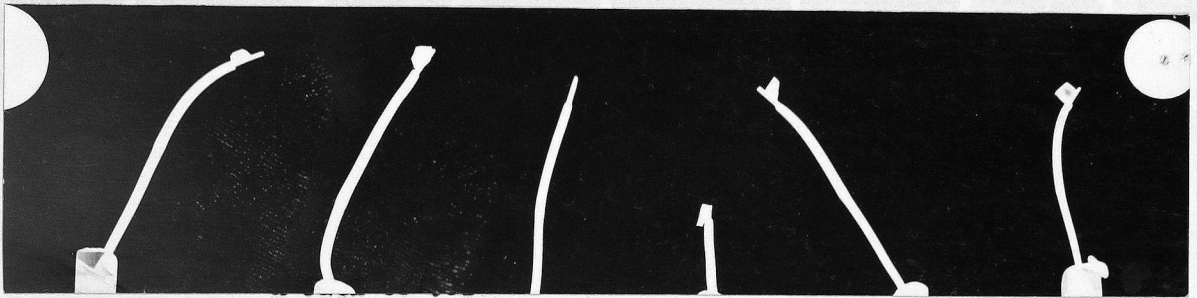
A rack of .1 Oct
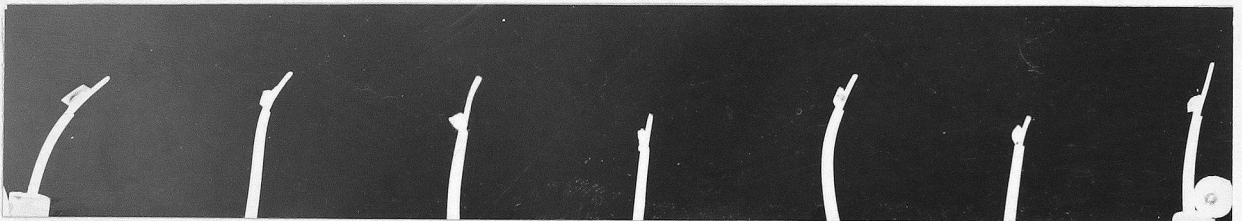
A rack of .01

A rack of .001
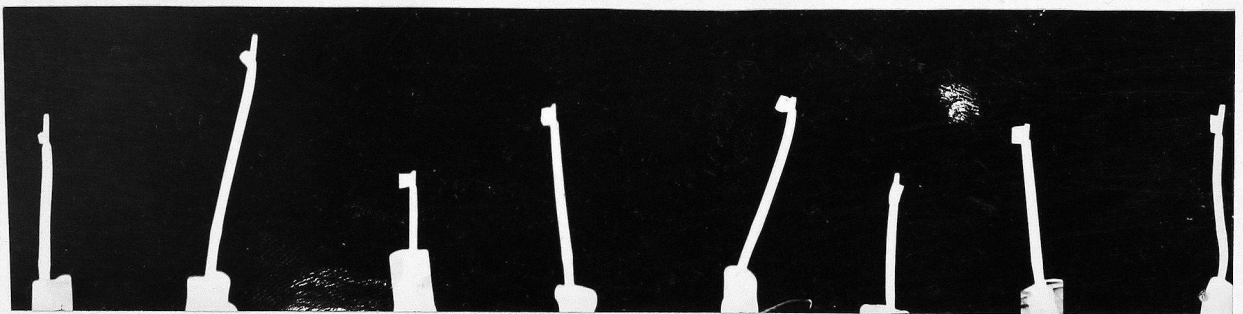
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |

A composite rack of 1 - 8, .001; 9 - 12, .01; 13 - 15 controls.
Taken two months after others. Notice that .001 is now apparently inactive.

Calculating Strength of Solution and Degree of Inactivation.

9	10	11	12	13	14	15
.01	.01	.01	.01	.00	.00	.00

means 80,000,000 A.E. per mg., which means we have at least 1 mg. equals .01 CC. . According to Rogers here is 1 gram in 100 liters of urine this will represent 1/200 of the original amin a 10 cc batch. Now if original 15 litres had a growth factor content of 30 mg. of amin a 1 mg., and if amin a had an activity of 30,000,000,000 A.E. per gram then 15 litres of raw material should have an activity of .03 times 30,000,000,000 A.E. equals 1,800,000,000 at a calculated yield of 15% then we should get an activity of 225,000,000 A.E. for a total . Inasmuch as the inactivity of our concentrate is about 3,000,000 A.E. then we might assume self-inactivation had cut it to an extent of 100 - (3,000,000/225,000,000 x100) equals 97.7%.

It should be emphasized at this point that the figures mentioned in regard to the effect of amin a are not constant. The effectiveness of standard solutions of original crystalline material constantly varies and Rogers has observed two extreme values of 4 x 10 and 5 x 10 A.E. per g. Exactly what causes this large variation is still a question for argument.

The concentrate thus, had lost most of its activity due to self-inactivation but considering the long time it took to isolate it, the time spent on the test tube technique and the disadvantage

Calculating Strength of Solution and Degree of Inactivation.

If curvature visible at .001 concentration then in a 2 cmm. block ther must be at least 2 Avena units, if, according to Went (72h) diffusion is 58%. Then in 1 ccm. there would be 1000 A.E. and in the original concentrate there would be therefore 1,000,000 per cc. and therefore 5,000,000 A.E. in the 5 ccs. of original dilution. Kögl says (33f) auxin a has an activity of 50,000,000,000 A.E. per gram. This means 50,000,000 A.E. per mg.; which means we have at least .1 of 1 mg. equals .0001 g. If according to Kögl there is 1 gram in 500 leters of urine this will represent 1/300 of the original auxin a in 15 litres. Now if original 15 litres had a growth factor content of 30 mg. of auxin a (33g), and if auxin a has an activity of 50,000,000,000 A.E. per gram then 15 litres of raw material should have an activity of .03 times 50,000,000,000 A.E. equals 1,500,000,000 at a calculated yield of 15% (33f) then we should get an activity of 225,000,000 A.E. for a total. Inasmuch as the inactivity of our concentrate is about 5,000,000 A.E. then we might assume self-inactivation had set in to an extent of 100 - (5,000,000/225,000,000 x100) equals 97.78%.

It should be emphasized at this point that the figures mentioned in regard to the effect of auxin a are not constant. The effectiveness of standard solutions of original crystalline material constantly varies and Kogl has observed (33f) extreme values of 2×10^{10} two 9×10^{10} A.E. per g. Exactly what caused this large variation is still a question for arguement.

The concentrate then, had lost most of its activity due to self-inactivation but considering the long time it took to isolate it, the time spent on the Went test technique and the discouraging

picture confronted us after translating Kogl's artical on self-inactiv-ation (35) of auxin a, the results exceeded expectations.

Testing γ-3 - indole-butyric Acid for Activity.

10 mg. were weighed out, dissolved in 10 ccs. of water and solutions of .001, .0001, .00001 were made up in Erlenmeyers. Blocks of agar were left to soak in them and sets of coleoptiles were concurrently grown. The test procedure was conducted through and the indole-butyric was found to be active to a concentration of .0001 which represent activity of about 1/50 of β-indole-acetic and about 1/100 of auxin a.

Possible Improvements on the Test Technique.

Possibly the biggest problem in the Went test technique was to get the seedlings to fit properly in their holders. By coming loose or getting at an angle a rack of 15 seedlings would produce 5 or 6 good test objects. It was hoped that if the seat for the seed could be done away with by dropping the seed down in a well-like holder, unhusked, then the coleoptile might grow out from one end of the gloom and the root out from the other. As a matter of fact it came to the writer's attention, during some preliminary work with unhusked seeds, that about 80% of unhusked seeds sprouted with the coleoptile immerging from one end of the gloom and the roots from the other. The advantages were obvious. Fourty unhusked seeds might be scattered on wet blotting paper in Petri dishes to germinate. When the roots have grown 1 or 2 cm. long out of one end of the gloom, the coleopti le is emmerging from the other. Selected seedlings are dropped into holders with their roots hanging through into the water trough and left to attain the proper

height for decapitation. The seedlings might have to be turned to get

the conductive bundles in a lateral position, however this is the work of

but a minute or two. Compared to the time lost in husking, discarding and

resetting coleoptiles in their loose seats this is a real improvement,

on the previous technique and leads to straighter, more uniform coleoptiles

in firmer seats and a great saving of time and loss of test objects as

well as a much simplified holder.
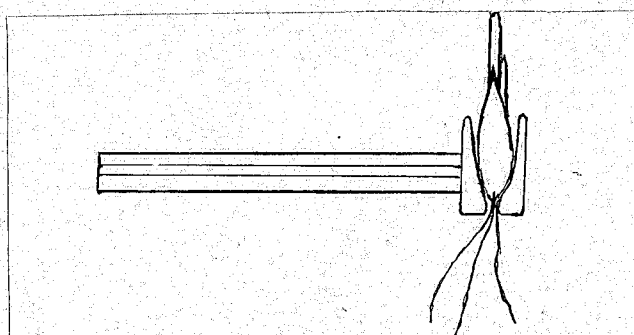
Figure 13.

The advantages are briefly summarized:

1. Holder can be made in $\frac{1}{2}$ the time.

2. Breakage of the seed seat is eliminated.

3. No time lost husking seeds.

4. Uniform coleoptiles can be chosen early as the gloom guides
   them the first cm. of their growth.

5. Space is saved by a reduction of the number of useless col-
   eoptiles.

6. No trouble adjusting endosperm in loose seats or trying to
   find a seat that will fit. The walls of the holder converge
   therefore nearly any one will fit.

7. No time or space lost resetting or replacing coleoptiles that
   have fallen out.

8. Since there are no losses of coleoptiles as the experiment
   proceeds all results are referable to common checks.

PART Ṕ.

Pretreatment of Seeds to Accelerate Germination.

That auxin might be playing a role in the physiological pre-
determination of seeds and their phasic development was a question that
answered either positively or negatively, could be considered contrib-
uting. Lyssenco (73) had an idea that plants in their development pass
through successive stages or phases. That our hormone and other active
factors might or might not play a role here seemed of considerable
interest and started us on some seed pretreatment work which ended up
with some interesting observations. It has been shown (see discussion)
that heteroauxin is in no way a germination hormone and if anything
retards it (5). It was a question if the auxin a was similar in this
respect, or might there be found a physiological difference between auxin
a and heteroauxin. The possible effects of varying concentrations of
urine in this respect and ethylene chlorhydrin were also investigated. It
might be added the attempt was not to break dormancy. It was believed
that was already completed and all the seeds needed for germination was
water. In any event it would be interesting to find out if these factors
effected seed germination. Heteroauxin and urine were used in prelimin-
ary work before auxin a was tried.

Preliminary Work-

Solutions of heteroauxin and urine were made up
in varying concentrations running to both extremes. Husked Avena seeds
were pre-soaked in these solutions for varying lengths of time, washed
and sowed crease down on 3% washed agar. The time element was controlled
by adding more seeds to soak every 24 hours and then removing them from
their individual vials, all at once, rather than taking them out every
24 hours. This meant we could compare them with one another and their

respective stages of development. In this way the time factor was
controlled. Apparently diffusion of active factors was complete in 24
hours because longer periods were without additional apparent effects.
It was found that both urine and heteroauxin inhibited germination
directly as the concentration. These results confirmed those of previous
workers in this field. (5/) It is also interesting to note in connection
with this experiment that both water and Kögl's dilution fluid controls
developed with no noticeable difference in one another as far as speed
of germination was concerned.

Pretreatment with Auxin a.

This experiment was preceded by trial work at
varying dilutions; controls of both water and Kögl's dilution fluid;
and vaseline sealed lids compared to cotton sealed lids which lowered the
humidity over that of the former. It was found in this preliminary work
that cotton sealed lids, leading to low humidity and possibly better
respiration was advisable; also that concentrations of 1, .5, .1, .001
and .0001 would be the most interesting. At dilutions higher than these
they were found to parallel the controls.

For this experiment 9 zoological specimen jars
were used. They were $\frac{1}{4}$ filled with agar solution and the washed seeds
rested crease down on the firm surface. The first seed in each jar
represented a Kögl's dilution fluid control. Other seeds in the same jar
would be from the same pre-soaking vial. A large 9th jar contained a
sample from each dilution and a water and Kögl's dilution fluid control.

cotton seal.
Avena.
agar.
museum jar.
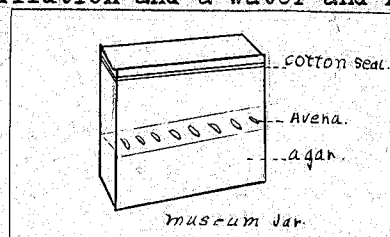
Figure 14.

The results were not photographed each 24 hours as we wished to omit the complicating light factor.

The seedlings were examined daily for four days. Those in jar 1 and 2 never did sprout. In jars 3, 4, 5 and 6 there was development varying inversely as the strength of the solution in which they were pretreated. There seemed at this stage little, if any, difference in the controls treated with Kögl's dilution fluid and water. It might be mentioned however the controls placed at the end of the row of seeds in each jar seemed to be slightly affected by auxin diffusing from auxin presoaked but water washed seeds, into the agar and thence into the control specimen. Also as time progressed to the fifth day the seedlings in jars 5 and 6 began to catch up with the controls.

It is believed that the inhibition found taking place on those treated at the higher concentrations was due to toxic influences and inhibition of root growth by the auxin, while that observed at the higher dilutions was due alone to auxin's known inhibitory effect on root growth. The following photographs will give the reader some idea of the stage of development of the different seeds about 50 hours after sowing.

Figure 15.

| Conc. | .001 | .01 | .1 | .5 | 1.0 | water control | Kogl's dilution fluid control |

After photographing, the seedlings were allowed to grow and daily examined four more days. At the end of this time the seedlings in jars 5 and 6 had apparently caught up with the controls while those in jars 1 and 2 were still either dormant or poisoned. The seedlings had now had a growing period of 170 hours and it was decided to soften the agar on which they had grown by placing the jars in a hot water bath. In this way the roots might be removed without breaking, from the agar in which they had grown, and measured to compare their length. The length of primary roots treated with the same concentration of auxin showed some variation but finding their average and comparing this from different jars showed some interesting results. The details of this comparison are tabulated below. Lengths of primary roots from jars 1 and 2 of course was 0.

No.	Conc. Pre-treatment	Kogl's control	Length of roots in different cases in cm.					Average	Aver. in extremes are disregarded
1	1		0	0	0	0	0	0	0
2	.5		0	0	0	0	0	0	0
3	.1	9.5	8.5	9.0	8.5	7.5	9.7	8.64	8.64
4	.01	10.0	7.5	9.0	11.2	8.5	7.4	8.70	8.70
5	.001	11.5	9.3	13.2	10.9	7.9	8.0	9.86	9.02
6	.0001	9.0	9.7	9.6	10.4	9.8		9.87	9.87
7	Kogl's dil. fluid	9.8	9.2	10.5	9.8	5.5		8.75	9.80
8	Water c.	9.3	10.10	8.5	10.9	11.5		10.25	10.25

The gradual increase in root length observed in the last column, according to the dilution seems to confirm our opinion of the stages of advancement of the different samples. One can see, from the extremes from the same jars, that accurate information would entail many test objects to be calculated in the average. It is believed by the author that the lower Ph. of Kogl's dilution fluid over that of the water control, was instrumental in liberating more active auxin to inhibit root growth in the case of the former, thus accounting for the variation in the average root length of the two controls.

A graph of root variation with auxin a pretreatment concentration

Pre-treatment concentration

Length of root in cm.
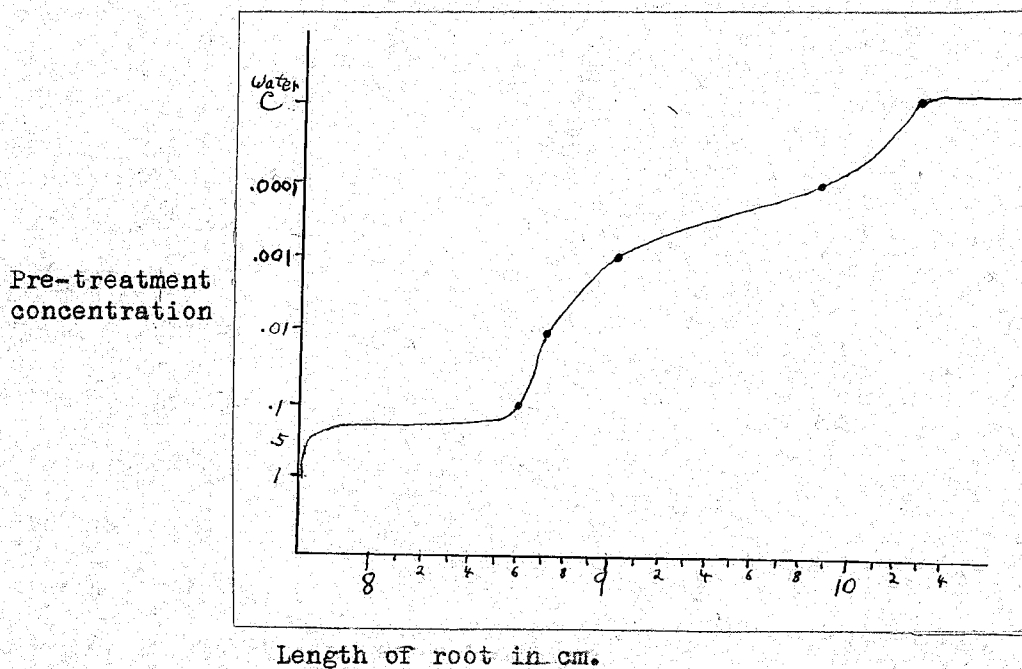
Figure 16.

This could not be shown with time as a variable because the roots could only be handled from agar once. However, we have good reason to believe it would look like this:
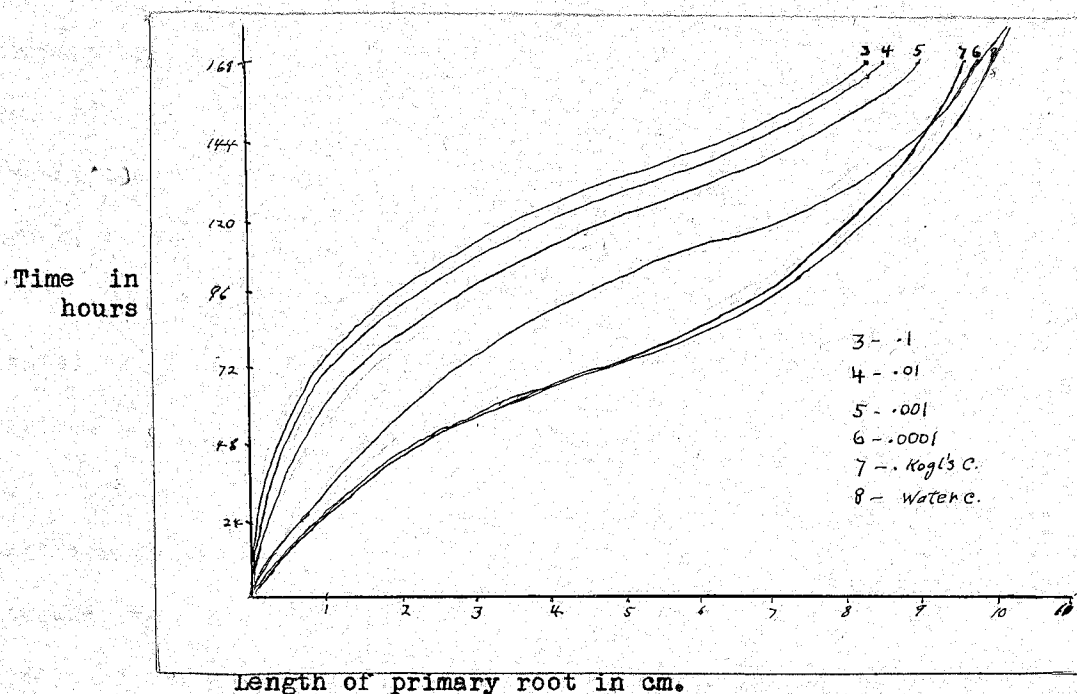
Figure 17.

Discussion of Figures 16 and 17.

Figure 16. There seems to be two dilution periods of improved growth.
The first appearing as plant comes out of a more toxic solution and
the second as the inhibiting effect of the auxin on root growth is
disappearing. Also the test object seems to show increased sensitivity
to small increases in dilution between .001 and .0001 concentration of
auxin a extract in the pretreatment.

Figure 17. This graph shows those pretreated at concentrations of .001
and .0001 catching up with the controls possibly due to the inhibiting
influence of auxin a on the root wearing off; whereas those from .1
and .01 concentrations are still much behind.

Pretreatment with ethylene chlorhydrin Vapours.

It was realized from the work of Denny (18) at
the Boyce Thompson Institute, that ethylene chlorhydrin vapours had

dormancy breaking and root promoting ability. Some curiosity arose in our minds as to whether it would have any effect on seeds which had already completed their dormant period and awaiting only moisture to sprout.

To satiate this question Avena seeds were pre-treated on the 26th of November in an atmosphere of ethylene chlorhydrin. This was supplied by suspending a piece of cheese cloth presoaked with 5 ccs. of 40% ethylene chlorhydrin in a museum jar. The controls were treated with 5 ccs. of water soaked into cheese cloth and suspended in a second museum jar. From this atmosphere the seeds were removed on November 31st. and sowed on wet blotting paper. The effect of the pretreatment on the length of the coleoptiles and the primary roots was striking and if properly interprexted might lead to something. The results of the measurement of the roots and coleoptile are tabulated below.

	Length in Cm.									Aver.	Med.	Ratio of Coleoptiles to root	
Seedling No.	1	2	3	4	5	6	7	8	9			Cal.on Aver.	Cal.on Med.
Length of Coleoptile	9.4	9.6	9.1	9.4	8.6	9.5	8.5			9.2	9.4	.75	.76
Length of P. Root	11.5	12.3	13.0	13.7	11.3	12.5	11.3			12.2	12.3		
Length of Coleoptile	8.2	9.2	8.6	7.6	6.5	8.5	8.6	7.0	7.8	8.0	8.2	1.06	1.05
Length of P. Root	5.8	8.6	6.8	6.5	8.7	7.8	8.4	6.5	8.3	7.5	7.8		

(Row groups labelled at left margin: "Ethylene" for the first pair, "C" for the second pair.)

It is significant to note that in the controls the coleoptiles were longer than the primary roots; whereas in the case of the ethylene pre-treatment the coleoptiles were shorter than the primary roots. Moreover the ethylene pre-treatment stimulated both the roots and the

coleoptiles over that of the controls.   Just what the position of cause

and effect is in this case is hard to say, as is the full significance

of the results.   However the ethylene chlorhydrin pre-treatment has brought

about a definite stimulas in growth of over 38%.

PART G.

Auxin a and Animal Tissue.

The question that the plant hormone auxin a might play a role in animal tissue has interested us greatly from the start. However it was not until the author became accidently innocculated by a dilution of his concentrate did this interest take an active turn.

In the course of pressing in a cork in a small vial held between the thumb and second finger the bottom broke out of the vial and the glass cut the tissue of the thumb permitting some of the auxin a concentrate .01 to gain entrance. Although the area injured soon healed over and gave no discomfort, it was noticed that a small tumor was appearing, which increased in size up to a certain point and then apparently remained dormant. The object, in this case, never before had had a tumor-like growth of any kind and there is no doubt in his mind that it was due to the concentrate accidently innocculated. After a period of four months, during which time it underwent no change in appearance, the author feeling that his utility as a test object had gone as far as possible decided that it would be interesting to cut it out, make a microscopic examination of it, and compare this with a malignant epidermal cancer that might be obtained from the Vancouver General Hospital.

Dr. Gee of the pathological department of that institution was consulted and she advised that being possibly malignant it should be removed along with any surrounding tissue as soon as possible. To this end she made arrangements with the emergency ward surgery where Dr. W. O. Green removed it with considerable adjoining tissue and closed the apperture with 3 stiches. At time of writing

the scar has well healed and apparently no regeneration of tumorous tissue has taken place. The following is our macroscopic and microscopic report on the tumor.

Macroscopic.

The specimen consisted of a small wedge of skin and subcutaneaus tissue, measuring 1 x 1 x 0.5 cm. on the superficial surface of which, is a warty, rough, slightly raised area, 0.5 cm. in diameter. On section this is seen to be situated entirely in the epithilium, but to be a papillary type of growth, firm in consistency, greyish-white in colour and apparently benign.

Microscopic.

Sections through the small growth showed it to be of typical papillomatous type, with fairly course broad based papillary projections consisting of well-defined uniform squamous epithelial cells. The basement membrane was intact throughout and very extensive keratinization appeared in the superficial layers. There was no evidence of malignancy and it would be diagnosed as hornifying papilloma. Following are some photographs of misroscopic slides prepared both from a malignant external growth on the cheek, and from it.
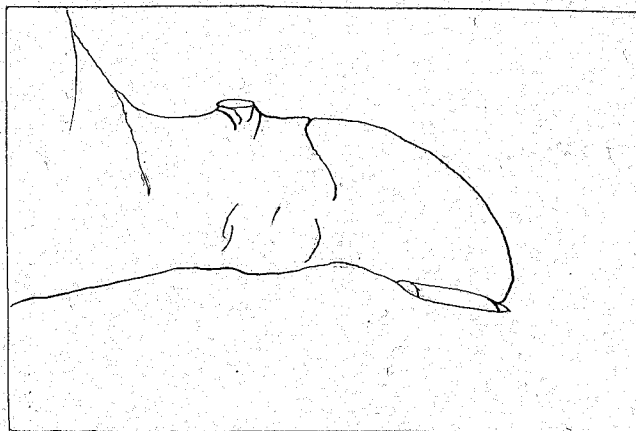
Figure 18.

Discussion on Microscopic Photographs.

A. Tumor from the Thumb.　Fig. 19 & 20.

Under low power basement membrane is shown to be quite intact. Under high power good cell differentiation and uniformity is shown and mitosis is relatively dormant.

B. Cancer from the Cheek.　Fig. 21 &22.

Under low power isolated islands have broken through the basement membrane and appear to be growing into the subcutaneous tissues. Under high power cells are not uniform in size or shape, differentiation is poor and deep staining is noticed. Mitotic figures can be observed and there seems to be a condensation of nuclear material.

TUMOUR FROM THE THUMB (LOW POWER).

Figure 19.

Fig. 19 cont. *38C* Tumor from the Thumb (low power)

TUMOUR FROM THE THUMB (HIGH POWER).

Figure 20.

Fig. 20 cont.   38e
Tumor from the Thumb. (high power).
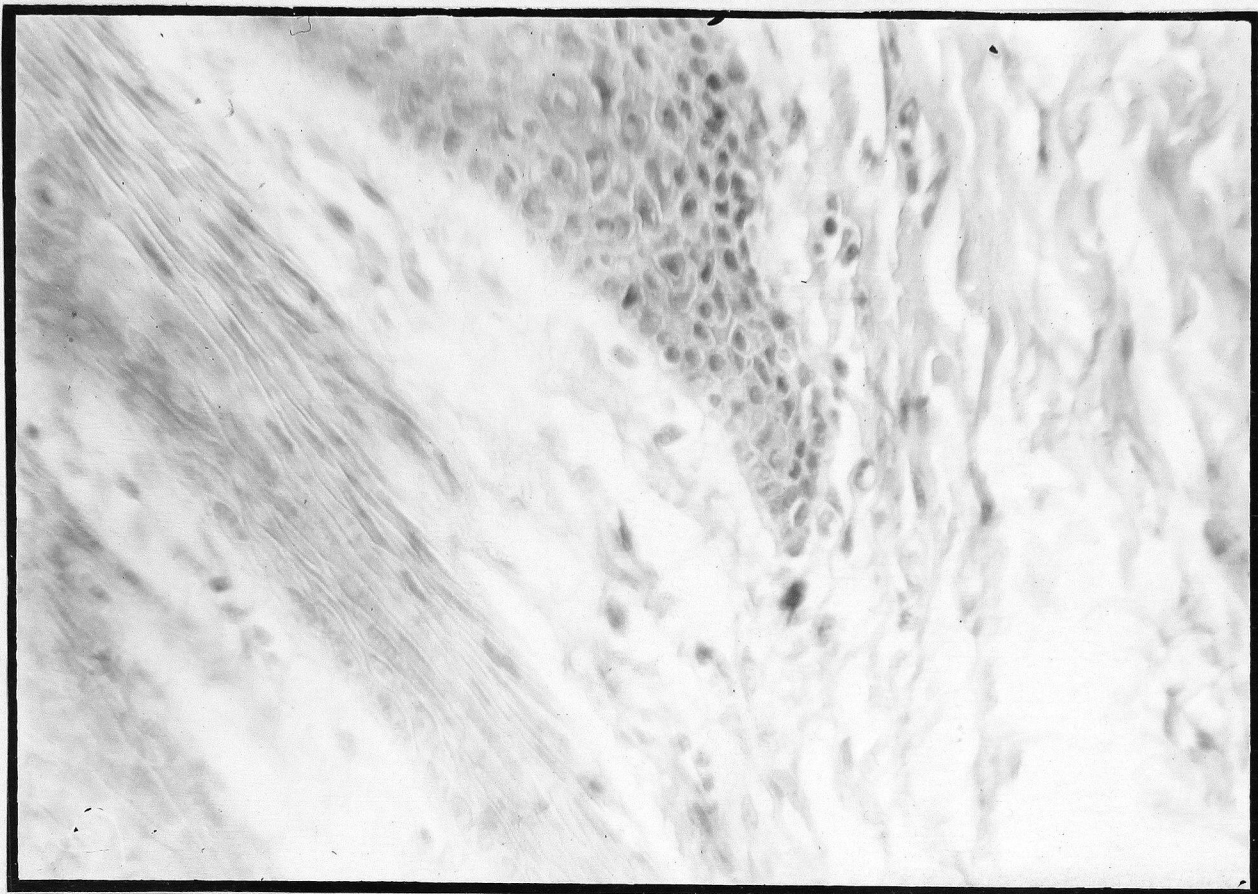
Fig. 21.   Cancer from the Cheek (low power)
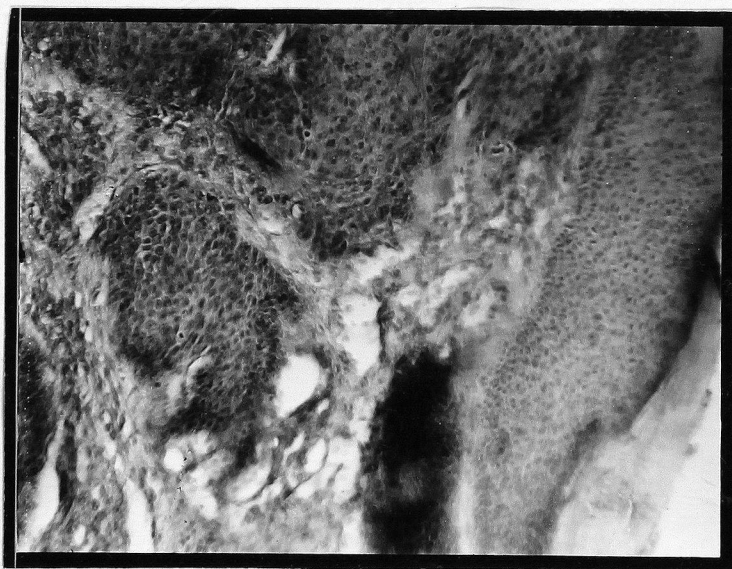
Cancer of the Cheek. (high power).
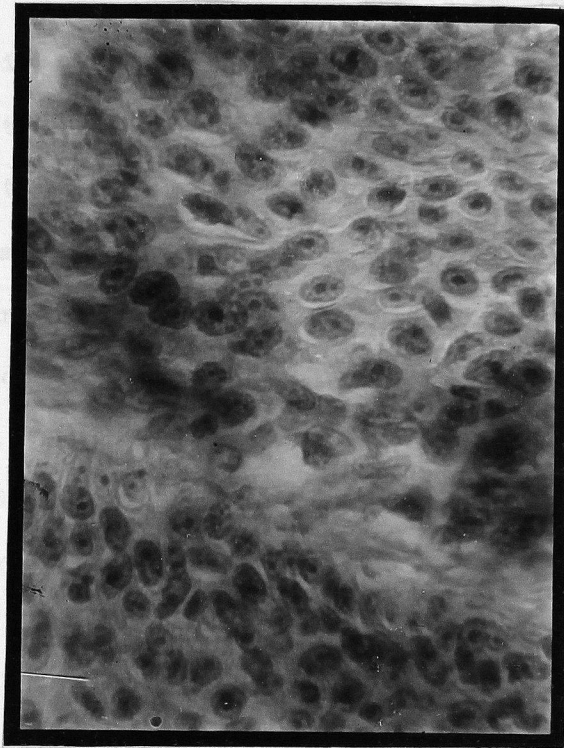
Fig. 22.

Treatment of Cuttings with Hormodin-A to stimulate rooting.

In view of the wealth of literature and public as well as scientific interest that has appeared recently regarding the rooting of cuttings it was decided to familiarize ourselves with procedures in this field and possibly make some contributions to the knowledge acc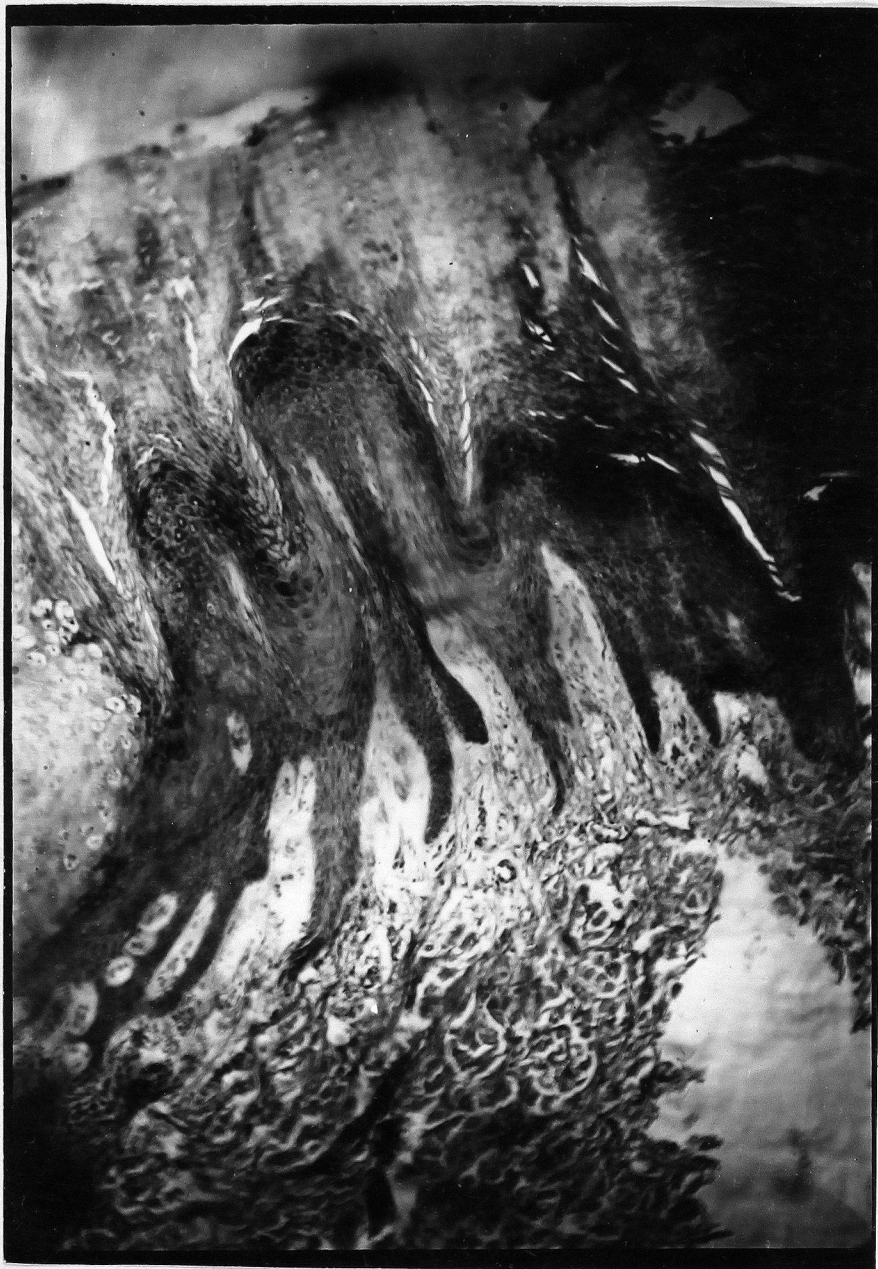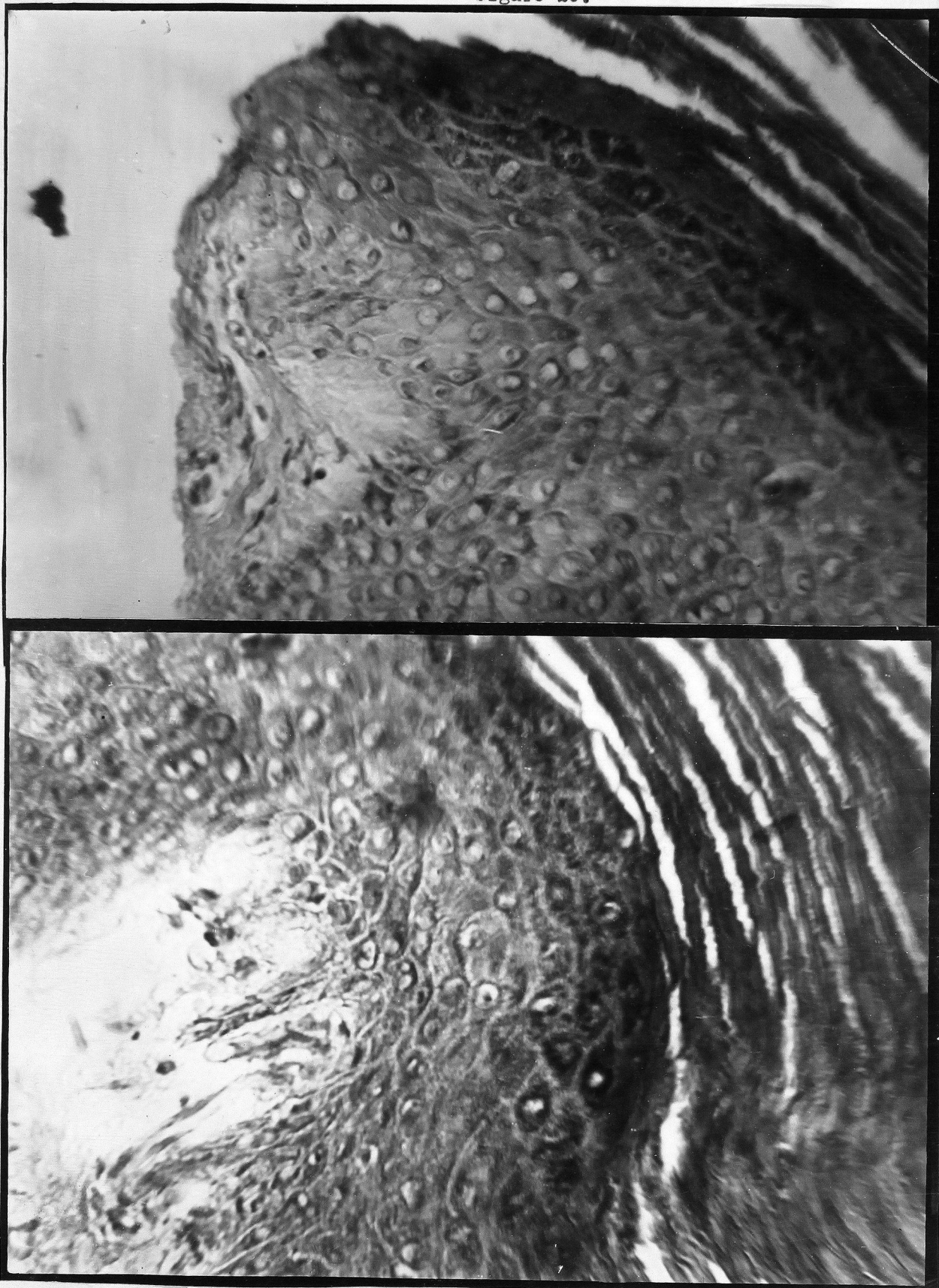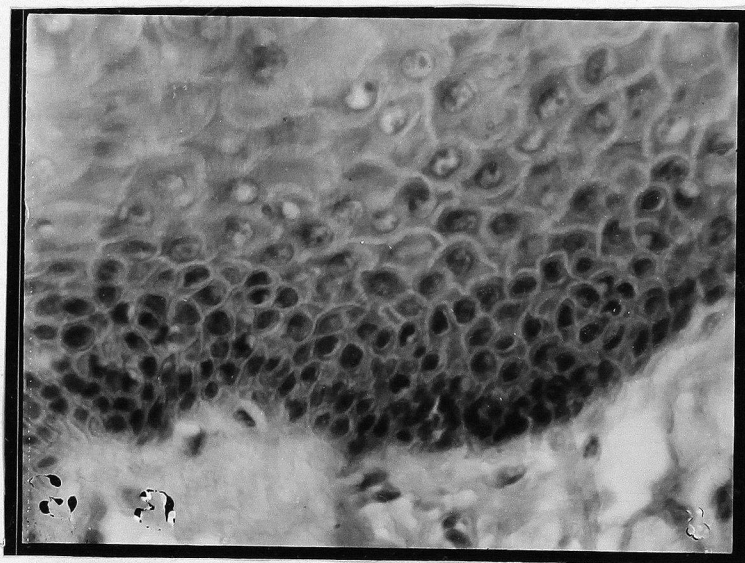umulated to date. As a matter of fact our interest received its initial impulse when we realized from the literature (64) that auxines not only caused cell stretching but stimulated the activity of the cambium layer. There was some notion in the mind of the author that auxin might cause stumps of freshly cut conifers to callous over and produce one or more sapplings one of which would eventually take the lead.

From this rather far fetched idea we modified our experimental ambitions to try and benefit the reforrestration of conifers in a more practical way viz. to promote the rooting of cuttings of one year old laterals by presoaking the basal end in varying concentrations of a commercial preparation of root promoting substance called hormodin - A. This experiment was undertaken in the Fall of the year and inasmuch as we were, at the time, also interested in Lyssenk's vernalization theories and the breaking of dormancy we decided to pretreat the cuttings at low temperatures and with ethylene chlorhydrin vapours. (2)

Lateral cuttings of Pseudotsuga Taxifolia 1 year old were placed in the ice box at freezing temperature for one week after which they were removed and subjected to ethylene chlorhydrin vapour (B.P. 120 C). The cuttings were placed in a closed container in which was suspended a piece of cotton containing 5 ccs of 40%

ethylene chlorhydrin.

In 5 days the cuttings were removed with a marked loss of needles. They were treated with dilutions of hormodin - A varying from 100, 80, 40 to 20 units at from 24, 48 to 72 hours.

From this point they were removed to flats in the greenhouse. The medium in which the ends were buried was a mixture of sand with a little peat moss to hold the moisture. In March an accident, during which the cuttings were accidently destroyed, befell this experiment.

Cascara Rhamnus cuttings, that were being treated simultaneously with the fir were lost at the same time. At the same time of setting out these last cuttings we (60) also treated a large variety of species with hormodin - A alone, some of which are now looking very promising.

Some 19 different species of ripewood cuttings were treated at concentrations of 40 to 20 units of hormodin - A for periods of 24, 48 and 72 hours and then placed in cold frames. The results are tabulated below.

A report on results with cuttings treated with
Hormodin - A will not be available until the third or
fourth week in May, 1938.

Species	No Treatment	40 Units			20 Units		
		24hrs.	48 hrs.	72hrs.	24	48	72
Pseudotsuga Taxifolia. (Douglas Fir)							
Chamaecyparis Nootkatensis. (Yellow Cypress)							
Juniperus Scopulorum. (Juniper)							
Taxus Brevifolia. (Yew)							
Abies Lasiocarpa. (Alpine Fir)							
Tsuga Mertensiana. (Blue Hemlock)							
Echinopanax Horridus.							
Cornus Nuttallii. (Fl. Dogwood)							
Rhododendron Californicum. (Rhododendron)							
Arbutus Menziesii. (Arbutus)							
Vaccinium Ovalifolium. (Blueberry)							
Shepherdia Canadensis. (Sopalallie)							
Quercus Garryana. (Oak)							
Jaglans Caneria. (Walnut)							
Fagus Sylvatica. (Beachnut)							
Liquidambar Styracflua. (Liquidamber)							
Daphne Cnedrum. (Pepper)							

DISCUSSION

Chemical Nature of Auxins.

To date there have been isolated 3 auxins. These are auxin a, auxin b, which is not the lactone of the former, and hetero-auxin or what is sometimes called indole - 3 - acetic acid. By the brilliant researches of Kogl, Erxleben and Haagen Smit (1933 and 1934) their constitutional formulas were elucidated. Auxin a and auxin b gave the greatest problem in this respect inasmuch as their total supply of available crystals was 700 mg. An example of their line of reasoning is presented below.(36)

First the acid and the lactone were shown to have but one double bond and the acid to have but one COOH group. After addition of hydrogen at the double bond the number of H. atoms in the molecule is still 2 short of saturation and hence there must be one ring in the molecule. In auxin a the remaining three oxygen atoms were found to be in hydroxyl groups, while in auxin b one hydroxyl and one keto group could be identified. Oxidative degredation of both auxin a and b gave rise to a C_{13} dicarboxyllic acid which contained no hydroxyl groups. Similar oxidation of the hydrogenated derivative which is biologically inactive yielded a neutral C_{13} ketone. The oxidation has therefore carried away all the hydroxyl groups, together with a chain of 5 carbon atoms. From the difference between the two oxidations it is also clear that the double bond was not in the side chain which was removed. Further reasoning indicated that this side chain contained the 3 hydroxyl groups and the COOH group extablished their relative positions, hence the oxidations must be formulated as follows, substance 1 being auxin a and 2 being dihydro-auxin a.

C.CHOH.CH$_2$CHOH.CHOH.COOH

(1)

COOH
COOH

(3)

CH

CH.CHOH.CH$_2$CHOH.CHOH.COOH

(2)

CO
CH$_2$

(4)

CH$_2$

C.CHOHCH$_2$COCH$_2$COOH

(5)

CH
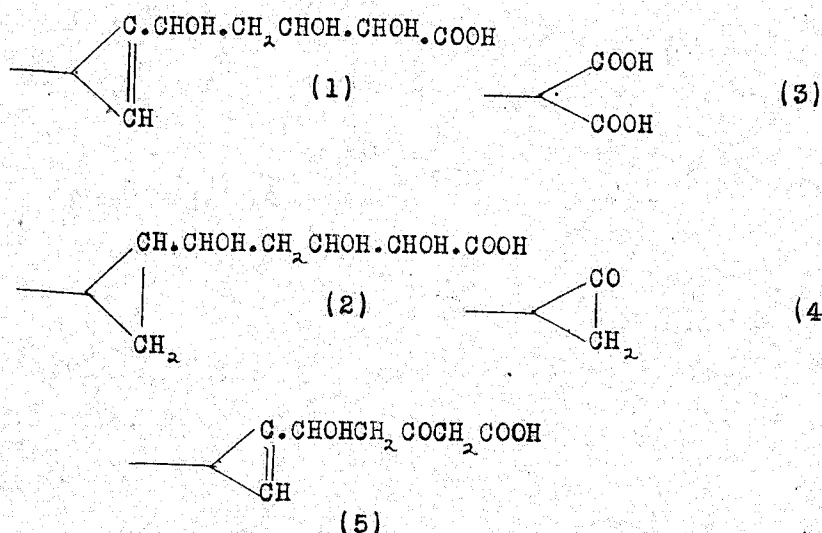
Since auxin b loses CO$_2$ on heating to gain a neutral ketone, it must have its ketO group in the B- position and therefore be formulated as 5. The relation between these two compounds, auxin a and b, or what is sometimes called (Kögl) auxentriolic and auxenolonic acids, is thus similar to the relation between the two female sex hormones, theelol (trihydroxyoestrin) and theelin (ketohydroxyoestrin). The structure of the C$_{13}$ residue was worked out by breakdown experiments while finally the synthesis of a dicarboxylic acid identical with the oxidation product 3 ("auxin-glutaric acid") (Kögl and Erxleben, 1935) (37) confirmed the following formula for auxin a and b.

CH$_3$

C$_2$H$_5$——CH——CH——C——CHOH.CH$_2$CHOH.CHOH.COOH

CH$_2$ auxin a, auxentriolic acid.

C$_2$H$_5$——CH——CH——CH

CH$_3$

CH₃
|
C₂H₅ —— CH —— CH —— C —— CHOH.CH₂COCH₂COOH
| ‖
CH₂   ‖   auxin b, auxenalonic acid.
|
C₂H₅ —— CH —— CH —— CH
|
CH₃
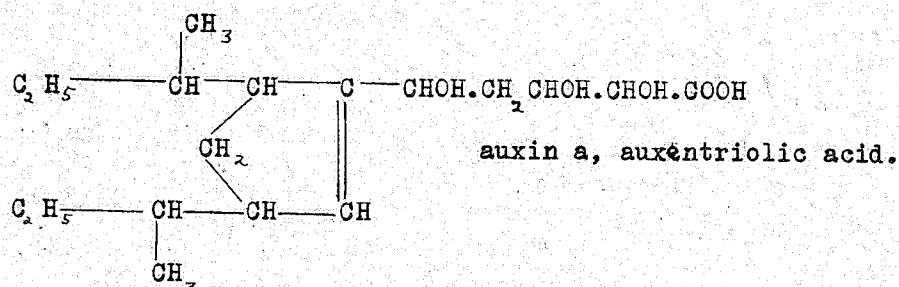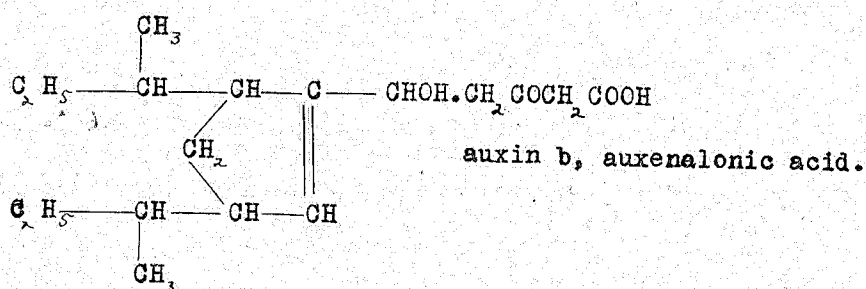
Their Self-inactivation. (35)

As mentioned in an earlier part of this paper both
auxin a and b have the unfortunate faculty of undergoing self-inactivation.
It occurs in the solid crystalline state most rapidly and involves no
oxidation because it can take place in vacue and in the dark, being
complete in 1 to 2 months. It was fortunate for us that when we had to
leave our work in March 1937 for 2 months that our isolation procedure
had not taken the product to too concentrated a state. Analysis of
inactivated auxin ("pseudo-auxin") showed no change in composition or
molecular weight, so the change must be one of isomerization. Study of
the ultra-violet spectra (Koningsberger, 1936, Kogl, 1936) (44) (35)
has shown that it consists of a shift of the double bond from the ring
to the side-chain, the γ - OH group shifting to the ring, this produces
an asymmetric C atom, which explains the formation of two optically isomeric
"pseudo-auxins" from auxin a:

C₄H₇·CH —— C —— CHOH CH₂ CHOH.CHOH.COOH
| ‖
OH₂   ‖   auxin a (active)
| ‖
C₄H₉CH —— CH

C₄H₇CH —— C ══ CH.CH₂ CHOH.CHOH.COOH
| |
CH | Pseudo-auxin a (inactive)
| |
C₄H₇- CH —— CH
|
OH
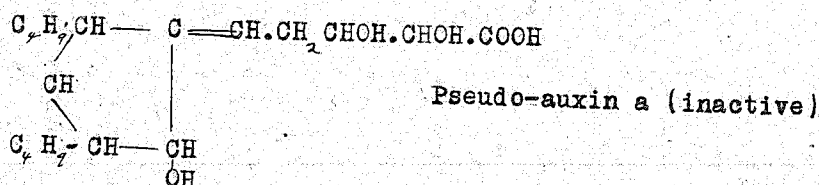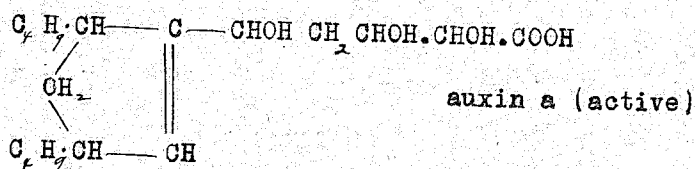
It is as yet unknown if this change has any physiological significance. In the case of auxin b, the lactone, the change is accompanied by loss of H_2O, and introduction of a second double bond in the side-chain, the same change may be brought about by ultra-violet irradiation.

It was not long after this work with auxin a and b that they discovered a substance in urine and in cultures of lower organisms which had physiological activity like that of auxin a and b, except that it was only about half as active. They found this substance to be identical with indole - 3 - acetic acid, and thus the formula for the third auxin, heteroauxin is:
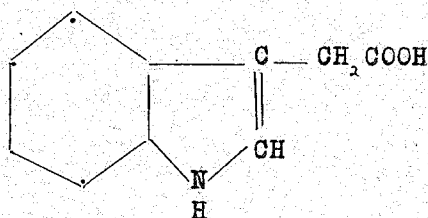
Its mode of formation in cultures by lower organisms is almost certainly by the oxidative deamination of tryptophane.

Relation Between Structure and Activity. (721)

Tables of all the substances which show any kind of activity on avena curvature, straight growth tests and pea stem curvature were drawn up by Kögl and Kostermans,(1935) (43) and tentative conclusions have been drawn which might suggest that:

Primary growth promoting activity (see Mechanism of Action, later) is connected with the presence of:

1. The double bond, or aromatic unsaturation.

2. A carboxyl group, free, or if esterified readily hydrolyzable.

3. A ring system, either 5 - membered (auxin a and b), aromatic (napthyl or phenyl), or a combination of both (indole, indene, etc.)

4. A minimum distance of at least 1 C atom between the carboxyl group and the ring.

5. A very definite steric structure, since in the one case studied the cis-compound is active, the trans-compound not.

The chemical changes which do not directly influence the primary growth reation, but modify the secondary properties (see mechanism of Action later) of a growth-promoting substance, are:

1. Length of the acid side-chain.

2. Methyl substitution in the nucleus.

3. Substitution in the side-chain.

4. The structure of the nucleus itself.

The Mechanism of the Action. (Primary and Secondary Properties)

The physiological activity of a substance depends not only on its direct effect but also on its secondary properties which determine whether it will reach the place of action or not. To use the simile of the key in the lock, the effectiveness of the key is determined not only by the wards but also by the grooves in the side of the key, which in themselves, have nothing to do with the opening process. Still, the incorrect arrangement of the grooves may prevent an otherwise correctly shaped key from opening the lock. The importance of this simile is then that we might study separably each property of the key, and are warned against ascribing the activity of a substance to those details in its chemical structure which affect only its secondary properties.

For example the rate of transport of a substance limits its activity. Thimmann (1935) (67) has shown that some substances inactive or of only small activity on Avena may be highly active in causing pea stem curvatures. (Another method of measuring cell elongation (72d)). Such substances may be regarded as possessing primary growth-promoting activity. Would it be possible then to test for the wards of the key independently of the grooves that is to test for a primary property of growth promotion without being confused by interference of others.

The relation between molar concentration and activity on pea stems has been compared for a number of substances which show widely varying activity in the Avena test. The result is that auxin a, indole - 3 - acetic, indole - 3 - propionic, indole - 3 - butyric, and γ - naphthalene-acetic acids at low concentrations approach the same activity per molecule. This means that irrespective of secondary factors which operate to decrease the activity on Avena the number of molecules of these substances necessary to produce a minimum growth effect is the same. The production of the primary growth effect is therefore, apparently, a chemical reaction involving stoichiometrical relationship between the active substance and the substrate inside the cell on which it acts. Substances which do not give the same molar ratio are presumably prevented from doing so by other secondary properties; these may or may not be the same as those which decrease their activity in the Avena test. A few of these secondary properties might be:

1. Transport. Its path and the forces which cause it are vague and holding back knowledge in this respect.

2. Permeability. Referring to the ability of a substance to enter the cell and reach its point of action. Its solubility in both water and lipoids probably plays an important part.

3. Sensitivity to inactivation. It is known that auxin a and b are

more sensitive to inactivation than heteroauxin, by oxidases.

4. A number of other factors such as lateral transport and the velocity of the actual growth-producing reaction, may also be of importance.

Identity in Higher Plants.

The reader might wonder which of these three auxins is the native growth hormone in the various higher plants. While this can be proved only by inumerable isolations Kögl, Haagen Smit and Erxleben, (1934) (40) have given good evidence by indirect methods that the active substance of the Avena coleoptile is auxin a. Firstly by calculating the molecular weight by diffusion methods (agar blocks).

The auxins from the higher plant sources by this method give a value of 328, while those from yeast and Rhizopus cultures give values of about 175. Secondly the sensitivity to pH provides a differentiation of another type; indole derivatives in general are destroyed by warm acid, but not by alkali; auxin a is destroyed by alkali but not by acid; auxin b is destroyed by both. The auxin of coleoptiles is sensitive to alkali and that from fungi to acid. The facts therefore are consistent with the view that the auxin of higher plants like Avena coleoptiles is auxin a; therefore it is indeed highly probable that other higher plants are stimulated by auxin a, in view of the fact that auxins have been shown not to be a species specific.

Nomenclature Inconsistencies.

Auxin a and b and heteroauxin are the only three substances to date that are known to exist in the plant kingdom and have cell stretching ability. Many synthetic preparations have been shown to permit or cause cell stretching. In the present condition of the

nomenclature because they give a reaction in the standard Went test it is permissable to call any of these synthetic chemicals auxins. In the opinion of the author they shouldn't be called auxins unless occuring naturally in the plant kingdom.

The Activity of Some Synthetic Compounds related to Indole-3-acetic Acid.

Since it was discovered that the common indole-3-acetic acid was active and occurred widely in the plant kingdom, Kögl and Kostermanns (1935) prepared a large number of derivatives of it, synthetically, and tested them by Avena curvature methods. There activity was found to be much less than that of the original heteroauxin. In their derivative research Kögl and Kostermanns first considered the importance of the carboxyl group and therefore prepared a series of esters. It was found that their activity decreased with increasing size of the esterifying radical. Kögl beleives the esters are hydrolyzed in the plant and that they owe their activity to the acid produced from them. The decreasing activities may be ascribed to decreasing rates of enzymic hydrolysis. The double bond appears to be essential for activity, for the 2 to 3 dihydro-derivative like the dihydro-derivatives of auxin a and b is inactive.

Numbering of ring-atoms of indole compounds is as follows:
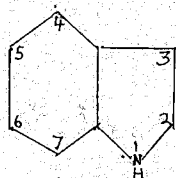
Introduction of an alkyl group into the 1 or 5 position of the nucleus always reduced the activity, sometimes completely. Methylation elsewhere (except for straight growth of Avena), while introduction of an ethyl group is more inactivating than methyl. Introduction of methoxyl in any position completely removes all activity. (72k)

The length of the acid side-chain also greatly affects the activity. For example indole - 3 - carboxylic acid is completely inactive as is also indole- 2 - carboxylic acid. Indole- 2 - propionic acid has only very slight activity, but indole -3 - isopropionic acid was almost comparable with indole-acetic acid. Of other derivatives indole-lactic acid was found inactive while indole pyruvic acid had low activity. In our own case of course it was found that indole - 3 - butyric acid was active.

Indole - 3 - alanine (trypophone) has slight auxin activity but this is lost on recrystallization and is therefore due to impurities. However, Went finds the behavior of tryptophane interesting from another standpoint. If applied to Avena curvatures, he says, are produced after a lapse of two hours or so. It also accelerates the growth of coleoptiles when applied to the base. (72k) Indole - 3 - ethylamine (tryptamine) behaves in the same way. (Skoog,1937) (62) The latter substance while it does not contain an acid group could be doubtless oxidized to indole -3- acetic acid, via indole -3- acetaldehyde. Tryptophane, as we have seen may be converted to indole -3- acetic acid by fungi and bacteria and the same conversion by plants is therefore extremly probable. Went feels that tryptophane and tryptamine are of special interest as constituting models of the auxin pre-cursor in the coleoptile: they are substances which, inactive in themselves, are readily converted to the active form by plant enzymes. Went also points that tryptamine is a base and therefore according to the electrical polarity theory (72l) would travel upwards in the plant. This would be very interesting if it had not been shown (72m) that the auxin produced in the growing tips of these higher plants was not indole -3- acetic acid.

Not only variations in the side-chain but
variations in the nucleus itself may be affected without wholly losing
the activity. Thus, Thimann (1935) (67) has shown that indene -3- acetic
acid which contains a carbon atom instead of the nitrogen of the indole has
moderate activity on Avena. On the other hand cumaryl-acetic, with
oxygen instead of the nitrogen atom was inactive in producing curvature.

Haagen Smitt and Went in 1935 listed (27) a
number af substances which they found to be inactive by all test methods.
Glover in 1936 reported that skatole had growth promoting activity on
Avena. Went has shown this to be false. (71n) Regarding the activity of
esters, it is of interest that the esters of auxin a are inactive. There
is good evidence that auxin a occurs in a number of natural products in
the inactive ester fomm. Thus Kögl, Erxleben and Haagen Smit (37) were
able to obtain considerable activity from several vegetable oils by
hydralyzing the oils with a lipase preparation or with sodium ethylate.
Some oils such as corn germ oil, contain auxin in free form. Auxin
esters in edible fats and oils may well be the source of the auxin a
in urine. This has been made very probable by feeding experiments. As
explained in a previous paper by the author (74) it was found that auxin
content in the urine increased greatly after ingestion of salad oils
and butter but not from ingestion of pure protein, sugar or hydrogen-
ated fats. (7d)

The Auxin Pre-cursor.

That the seed plays a part in the formation of
auxin in the coleoptile has been explained by Skoog (62). If the seed
is removed auxin production in the coleoptile tip decreases and if the

plant is decapitated and 2 hours later regeneration of auxin production
in the stump does not occur. The plants remain normal however because they
are still sensitive to applied auxin. The auxin secreted by the coleoptile
tip does not come directly from the seed as has been claimed by Phol in
1936, because if it did it should be possible to intercept it by removing
the coleoptile and replacing an agar block on the stump. Skoog could
obtain no auxin in this way, even with very young plants. This was shown
by the failure of such blocks to produce any curvature on the Avena after
3 hours. If however the blocks were left on the test coleoptiles for a
longer period they give rise to distinct auxin curvature, this indicates
that some substance (precursor) is being converted into auxin within the
tissues of the test plant. For such tests deseeded plants, which do not
show any regeneration were used. The evidence is therefore strong that
a precursor, present in the seed, moves up into the coleoptile tip and is
there converted into auxin. Some of it is doubtless also converted into
auxin in the seed itself. Cholodny has shown that wetting of seeds causes
rapid auxin formation. (9)

The free auxin thus formed is probably from the
precursor. It may be suggested (Skoog) that this precursor is an ester,
the esters of auxin a being themselves inactive.

In general the production of auxin takes place
in the apical parts of all organs which are actively elongating, such
as terminal buds on growing stems, young leaves on growing petioles,
infloresences or flowers on growing flower stalks and coleoptile tips.
Now we know in young seedlings the last stage in the production of auxins
can take place in the darkness (62) and since it might be concluded from
the general agreements between the experiments on plants other than

seedlings, that auxin production in such plants takes place only as a result of the action of light, it follows that light is necessary for the formation of the auxin precursor. (720) There must therefore be a definite light-sensitive reaction involved in the formation of the precursor. In 1928 Gregory (25) came to the conclusion that a master photo chemical reaction, independent of carbon assimilation, leads to the formation of a substance directly involved in leaf expansion. There is nothing in the literature to show that auxin itself plays any part in leaf expansion except that it is connected with the elongation of the veins. The mesophyll is not influenced at all by auxin application.

The cases on record where auxin is formed in the dark are all provided by seedlings. In these it is almost certainly formed from an inactive precursor which is stored in the seed. It is important then for the growth of seedlings which have to begin their lives in the darkness, not only carbohydrates and other nutrients but also auxin is thus stored in a readily available form.

Auxin production is not in general due to meristematic activity or to the presence of embryonic cells as is proven by its production in coleoptile tips and in leaves. Weight for weight, auxin production is the greatest in the very young leaves of the bud. (22) On the other hand there is a distinct correlation between the position of an organ and its auxin production, only the most apical regions form auxin, and the more terminal their position the greater the production.

Other Methods of Auxin Determination.

   Although the Went test technique of coleoptile
bending is universally accepted as a method of testing growth substances,
other methods have been devised which have certain merits in their favor
also. Among these are the Pea test, straight growth methods, epinastic
response and such variations of the Went technique as removing the seed
to increase sensitivity, or applying the hormone in lanoline paste instead
of by agar blocks. An excellent account of these may be found in
"Phytohormones" by Went, 1937. The pea test being most popular of these
other methods it might be well to mention it briefly.

   When elongating organs, particularly stems are
split longitudinally in the growing zone, the two halves curve outward
in water and inward in auxin solution. The outward curvature is due to
tissue tension, the epidermal cells being normally under tension and the
pith cells under pressure. The inward curvature is a differential growth
phenomena of a complex nature. (Jost and Reiss, 1936) (32). Such cur-
vatures in pea stems can be used as a convenient quantitative test for
auxin. This test has been shown by Thimmann (in 1935) (67) to be
useful for testing substances that may be active in growth promoting
without showing any curvature in Avena. For example Cumaryl-acetic will
not bring about curvature in Avena but w ill show stimulated cell elong-
ation in the straight growth test or cause curvature in the pea test.
That is, it apparently possesses the primary but not the secondary
properties as mentioned on page 47 of this discussion.

   In the straight growth method of auxin determin-
ation quite short cylindrical sections of coleoptiles are placed in
auxin solutions. They respond by an increase in growth which is prop-

-ortional, within limits, to the logarithm of the concentration of the
auxin (Bonner, 1933) (5). This makes possible a simple auxin determin-
ation by straight growth measurements under the microscope, eliminating
the necessity for humidity control. Distance of the sections from the
tip, residual growth and age of seedling all vary to make this test poor
for quantitative work.

B. A. Scheer (1937) (58) finds that at high
concentrations of g.s. Went's test is not accurate due to transverse
spreading of the hormone. They suggest another method of measuring the
overall length of the coleoptile. An agar block containing the G.s. is
placed squarely on the decapitated stump and the total length measured in
24 hours. With this method they obtained an increase in length of 100%
in 24 hours.

The convenience of curvature methods rests upon
two facts (1.) the residual growth, after decapitation is the same on
both sides of the plant and thus is automatically eliminated from the
measurements -- no controls are necessary; and (2.) only one measurement
need be made; there is no zero reading for the plants are chosen to be
straight. Besides this, the curvature magnifies the amount of growth
occuring and thus makes it easy to measure.

The fact that auxin is involved in a large
number of processes other than simple elongation makes possible a number
of means of assaying it. The inhibiting effect of auxin on the growth
of roots on cuttings, the inhibition of lateral bud development and the
production of swellings on stems and hypocatyls as well as its effect on
leaf petiols have all been used with more or less success.

On Germination of Seeds.

        Ethylene chlorhydrin has been shown (72b) to promote the growth of roots. Most substances having this auxin-like characteristic inhibit root lengthening. In View of this it is peculiar that the ratio of the root length of those pre-treated with ethylene chlorhydrin, over those of the controls is 1.58. In the seedlings pre-treated with the vapours, if the coleoptiles had been longer than the roots, it would compare more favorably with the controls and we might interpret our results as a straight stimulation of growth. However this is not the case. The roots have turned out longer than the coleoptiles which means they have been stimulated over and obove that of ordinary growth, inasmuch as the root coleoptile ratio in those pre-treated is 1.33 as compared to .90 in the controls.

        Van der Laan (1934) (45) reported that ethylene inhibited the formation of growth substances in Avena. More recent studies by Crocker, Hitchcock, and Zimmerman (1935) (13) suggest that ethylene itself may be a plant hormone, though the experiments of Michener 1935) (72.) have failed to show any growth-promoting effect of ethylene applied in a series of concentrations to Avena coleoptiles.

        Van der Laan (1934) (45) treated seedlings of Vicia Faba with ethylene, the greater part of the growth substance in them disappeared as did geotropic sensitivity, and the shoot grew horizontally.

        The author would like to suggest that in view of these results of Van der Laan, possibly when pre-treating our seeds with ethylene vapours we pre-determined the destruction of the auxin as far as it was concerned in inhibiting root lengthening; thus accounting for our

longer roots in the case of those pre-treated with ethylene. But this does not explain the increase in length of the coleoptile over that of the controls. To this end let us say to the extent that the root length might be a limiting factor in growth of the areal parts then the longer roots should produce longer coleoptiles. Just how the auxin might be destroyed as far as its root lengthening inhibiting qualities exist and yet be preserved for its cell stretching work in the coleoptile, brings us back to the old question of how it can stimulate coleoptiles and inhibit roots. Now Van der Laan (1934) (45) also showed that ethylene decreases the production of growth substance in Avena and in Vicia Faba but not its utilization, for, when a growth substance is directly applied, growth is not affected. Now in our case auxin is being produced at the growing tip of the coleoptile. This can promote cell stretching as it travels basally. That means it can be utilized, being freshly produced. This might account for our increased length of coleoptiles.

Crocker, Hitchcock, and Zimmerman (1935) (13) concluded that ethylene may itself act as a hormone. Went says the effect of ethylene on growth by elongation is to inhibit and not to promote. In Salix cuttings, ethylene alone has a small effect in increasing the numbers of roots, but if they are treated with auxin, its effect is greatly increased by ethylene treatment. Thus ethylene only seems to be effective in the presence of auxin. Since the experiments of Crocker, Hitchcock, and Zimmerman were carried out on green plants, rich in auxin it is highly probable that the action of ethylene which they observed, was through its effect on auxin already in the plant.

Seeds and Hormones and Ethylene

As has already been mentioned the results showing

an inhibiting effect on seed germination by auxins confirms these of
other workers in this field (W. Davies) (16), T. Solocalu) (65), 1937.
However Atkins and Hudson (1937) (17), give evidence to show that if no
stimulation in germination of seeds takes place on auxin pre-treatment
then acceleration of growth and length of shoots takes place later on.
The fact that our higher dilutions eventually caught up with the controls
confirms this.

Cholodny in 1936 (10) in an attempt to explain
vernalization in terms of auxin accumulation in the meristematic tissues
of the embryo, treated various grains with heteroauxin and found the
plants so treated flowered at the same time as their control but their
yield was increased.

Grace (23) treated tomato seeds with heteroauxin
and many of its derivatives incorporated in talc dust (Dosage 50 - 250
mg. per acre) and obtained a stimulation in root and top growth accuring
later.

W. Davies and others 1937 (16) report that all
compounds, tried except ascorbic acid had a retarding effect in any concentration
on the germination and subsequent growth of oats.

Experiments have been carried out (Nielson N. 1936 (53)
on the growth substance content of seeds of varying ages. It was found that
there was no appreciable variation. It was moreover found that germin-
ation capacity to be almost entirely inhibited in the older seeds. There
might then be no relation between germination capacity and content of
auxin.

Certain workers (Cholodny et al, 1937) (17)
believe germination is brought about by a hormone which they call

blastanin, changing from its pre-cursor state to an active state. It is theirbeleif that it passes from the endosperm into the embryo, and is thus the physiological cause of vernalization. Low temperature and re- duced moisture maintained during vernalization retard embryo growth owing to which, blastanin accumulates in the tip of the embryo and later accelerates plant development.

Ascorbic acid is known to occur naturally in the plant and as previously stated workers have found that it stimulates germination. Whether or not it is blastanin or is related to it is unknown. It cannot be termed an auxin as it does not cause cell elon- gation, (Clarke, W. G., 1937) (12), nor does it facilitate the action of auxin in the standard Avena or pea stmem curvature test. Ascorbic acid is not present in the germinating seed but is synthesized in the coleoptile from a precursor in the seed. Reduced ascorbic acid (also according to W. G. Clarke) is oxidized more by extracts from basal sections of the coleoptiles than by extracts from apical sections; this destructive gradient corresponds with the destructive gradient of auxins in the coleoptile and also might be associated with the fact that the distribution of peroxidases varies inversly to this; ie. their concen- tration gets greater as one proceeds basally.

Auxin a and Animal Tissue.

Seubert showed in 1925 that auxin may be present in high concentrationsiin saliva and pepsin, both animal secretions. Kögl, Haagen Smit and Erxleben in 1933 (33d) made systematic examinations of animal excretions and tissues. Most tissues contain very small amounts, the kidneys, and the colon and its contents having the most. Maschmann and Laibach in 1933 (53) found the liver and kidneys of the mouse and hen

to be higher in auxin than other tissues. Maschmann (1933) (53) and

Kogl, Haagen Smit and Tonnis (1933) (41) found the auxin content of

human carcinomas is higher than that of surrounding tissue.

Fischer found no effect of auxins on the growth of

animal tissue cultures (see Kogl, Haagen Smit and Tonnis, 1933) (41).

This however is not conclusive proof of the ineffectiveness of auxin since

the embryonic fluid which is used for tissue cultures is relatively rich

in auxin (72q). It is beleived the large quantity of auxin in the urine

is derived partly from the food and partly from the action of bacteria

in the intestine.

Robinson and Woodside (1937) (56) have followed the

auxin formation in the hen's egg during its development. The auxin which

is principally in the embryo increases parallel to the increase in weight

of the embryo for the first 7 days; thereafter it increases rapidly to

reach a maximum of about 40,000 plant units or 1 γ of auxin at about 14

days. Finally it decreases again. There seems to be a suggestive par-

allelism between auxin content and growth. Mavez and Kropp (1934) (34)

attempted to associate auxin with the chromatophore activator. Baysen

Jensen in 1932 (6) showed that certain amino-acids are particularly

favorable for the production of heteroauxin, the most of which was trypto-

phane, a constituent of peptones. It is believed that this is the source

from which bacteria produce heteroauxin, as a break down product of

tryptophane.

Went does not seem to favor the idea that auxin

plays any part in the animal kingdom although he concedes that auxin

production by pathogenic microorganisms may be of great importance in

plant diseases. He beleives that little comparison can be drawn between

the auxins and the animal carcinogenic substances. These substances, he

says, are hydro-carbons (Fieser, 1936) (20), are very slow in acting,

and it seems probable that their effect is to induce normal cells to

change into cancerous cells rather than to promote cell growth directly.

Auxins vs. Animal Hormones.

The rapid progress of work in the field of plant

hormones might be attributed to the relative simplicity of the relation

we beleive to exist between the plant and its hormones, as contrasted with

the apparent complexity of the same situation in animals. The harmonal

correlation between different parts of an organism will be the more comp-

lex the larger the number of parts; the plant, with its few organs is

thus a relatively favorable organism for study and should, in the opinion

of several eminant authorities in the medical fraternity of this city,

lead to a great enrichment of our knowledge regarding hormonal activities.

In animal the action of hormones is often indirect. The pituary exerts

an effect on the gonods vitamin D may act through the parathyoid, etc.

These additional links introduced, make the process harder to explain.

We don't maintain that in plants the process is itself any simpler but only

more open to attack; for example we can individuate between the primary

process in which auxin takes part from the secondary ones which prepare

for its action.

The hormones of the two kingdoms can be contrasted

in several respects. In animals the food and hormones all travel along

the same path, namely that of the blood stream. This means all cells

receive the same hormonal stimulus and the result will depend on their

ability to respond to it. Plants on the other hand have no true circul-

ation and the movement of hormones in them is mainly unidirectional, independent of the mass movements of water and foods. Thus hormones and foods move by different paths and hence every cell is not in a position to respond; this results in local growth zones and phenomena such as apical growth. (72r)

The hormones of the two kingdoms also have suffic- ient parallelisms to merit the use of a word which was fast coined for animal physiology. (Bayless and Starling, 1904) (72s). First the basic function of hormones, that of chemical messengers (Bayless), is the same in plants as in animals: -- our phyto-hormones then are truly hormones. Second, the activity is exerted in concentrations too dilute to allow of their making up an appreciable part of the cell wall or contents.

Regarding the specificity of plant and animal hormones, there seems to be very little species specificity in either case. That is one hormone performs the same function in a large class of organisms. On the other hand there is specificity of function, a feature which is more marked in animals than in plants. We beleive the auxins bring about a number of different effects, while the animal hormones seem to have one special function. The specificity of function in animal hormones is strictly dependent on their molecular structure so that in the sterols for instance, small changes in the molecule completely alter certain functions of the substance: hydrogenation of arromatic ring converts a female to a male sex hormone. In the auxins, according to Went (72t) small changes alter only the quantitative activity of the substance and do not change its function. Just how true this will be shown to be in the next few years is a matter of doubt. Quantitative changes in activity with small changes in the molecule also occur in the sex hormones. Whether these changes are due to differences in secondary properties, such as

penetration, partial inactivation, etc. as with the auxins has not yet been shown.

The author has shown the only known effect to date of plant hormones on animal tissue. There is certain evidence that animal hormones have an effect on plant growth. Oestrone in particular has been known to increase the dry weight production and flowering of a number of plants (Schoeller and Gaebel, 1935) (59). In some of Went's unpublished results he indicates that oestrone (theelin) promotes the growth of roots if auxin is present. (72u)

The idea that plant hormones could have any possible effect in the animal kingdom does not meet much favour with F. W. Went. His opinion is admittedly based on experiments conducted by Kögl et al where in tissue cultures en vitrio were treated with one of the auxins. In this experiment they used embryonic fluid for the cultures which has since then been shown to be rich in auxin. Also they might have used hetroauxin and not auxin a. This would decrease their sensitivity to applied auxin. We realize our results with animal tissue might be criticized on the basis of not having all factors under control, however it was not started as an experiment and only mentioned as possibly one of auxins interesting effects.

F. G. Gustafsen (26) has shown that parthenocarpy has been induced by pollen extracts. The extract was mixed in lonaline and applied to the stigma or cut pistil of pepper and eggplant. It was then found to promote the growth of ovaries and produce seedless fruits. Is it possible that chloroform or water extracts of sperms might not stimulate dormant ovaries in humans.

Plant Tumors and Their Relation to Cancer.

The crown-gall disease of plants can resemble only cancer of the skin and is best visualized as a warty, globular mass of tissue the surface of which is studded with a large number of smaller spherical boddies partially imbedded in the underlying mass of tissue. In other cases the gall is perfectly smooth with a thin layer of epidermis covering its outer surfaces and may grossly resemble a sarcoma of a long bone. As the disease appears in nature it is characterized by a smooth swelling or by an irregular, warty mass of tissue found on the surface of the stem above the roots. This region of the plant is commonly referred to as the crown portion of the plant, hence the name, crown-gall. Jensen was among the first to undertake an intensive study of the crown-gall. He concurred, in the main, that crown-gall resembled cancer. (3/)

Many bacterial parasites have been found associated with cancer but in no case has the investigator been able to establish the organism as the etiological agent. Bacteria as secondary invaders in cancer are well known. Levin and Levine (47) in a study of the analogie between the crown-gall and human cancer concluded that the reaction tissue in crown-gall may be best compared with the imflammatory processes in infectious diseases of animals. Devoid of cell types found in animals the plant is capable of reproducing only the cell that is injured, so forming a protective mechanism comparable in animals of scar tissue. It has been shown by Levine (50) that under conditions still unknown this reaction tissue may become extensive and invade the normal tissue of the host, producing a destructive new growth. Under these conditions, the crown-gall is comparable to malignant cancer of animals and man.

An essential characteristic of the malignant animal tumor is the production of similar growths, metastases, in parts of the body distant from the seat of the primary neoplasm. Metastases, or the dissemination of crown-gall by emboli to other parts of the plant, do not occur because of the structural characteristics of the plant. The movement of th fluids, blood, and lymph in the animal and the mature of the animal tissue favor, according to the accepted views of the present day, the separation of small clusters of cells from the main tumor and their transportation to distant parts of the body. Here cells become lodged andproceed to grow, forming secondary growth. This new growth consists of types of cells identical with those of the primary neoplasms. The plant fluid, sap, most likely passes through cell walls and cell pores, but particles as large as cells cannot pass through these walls even if they were separated from the primary crown-gall. The analogy between the neoplasms of man and animals and the crown-gall disease of plants does not hold in this important and fundamental point.

The layman's conception that malignant growths are characterized by the development of 'roots' has good foundation in the studies of Handley (23) and others. Malignant tissues form strands of cancer cells from the primary growth along lymph channels until a favoralbe location is reached where the growth takes on a nodular form. Smith, Brown and Townsend contended that crown-gall produces strands of crowngall tissue so that a new growth is formed from one already present. Levine made microscopic preparations of crown-galls from petioles and failed to find any connecting tumor strands. The tumor on the older part which becomes visible first has been referred to as the primary growth, while the younger gall, which becomes visible later, has been referred to

as secondary tumor. Metastases in crown-gall do not occur and the so-called secondary tumors are produced by the same inoculation that cause the primary growth. There is no tumor cell continuity between primary and secondary tumor over-growths in plants. The terms primary and secondary tumors in crown-gall represent a temporal relationship in the developemtn of two galls arising from simultaneous inoculations of embryonic tissues. In cancer it is believed that the secondary growths arise directly from the primary growth either by emboli or strands.

Berridge (4) on the basis of a study of the pH concentrations of the crown-gall tissue, contends that the pH concentration of the plant sap most favourable for the growth of Bacterium tumefaciens (an organism which can bring about crown-gall) is at 5.2. This pH concentration characterizes the meristematic tissue where the crown-gall arises. It is interesting to note that the auxins are also active at this pH.

It may be stated briefly that Smith(63) believed that bacteria were not necessary to produce crown-galls but that the products of the metabolism of the parasite were sufficient. The production of tumors in plants by chemical means has been the subject of considerable experimentations, but the reaction tissues so formed are small and do not reach the over-growth stage, although intumescences and internal cell proliferations of a limited type have been reported,(Levine 1936) (50).

Another aspect of the problem which widens the gap of analogy between cancer of animals and crown-gall of plants is found in the cytological phenomena of these two classes of over-growths. It has been known that cells in human and animal cancer tissues are abnormal in their behavior, irregular in size, and proliferate with very little

regularity. For this reason their cytology has been studied with some hope of throwing some light on the cause. A number of investigators of animal cancer have described atypical asymmetrical divisions, monaster and multipolar spindles, and amitotic nuclear divisions in cancer cells. Cells with a larger number of chromosomes than normal for the species were considered by von Hanseman and others to be due to an unequal distribution of the chromosomes in nuclear division. On the basis of these phemonema various theories as to the cause of animal cancer have been evolved. Some believed that cancer was due to fusion of somatic cells, altered chromosomal behavior, a somatic mutation, and that the cancer cell is an altered one and capable of perpetuating itself. Stillothers believe that some hormone, or other substance, is being concentrated in that area and causes proliferation of the cells so rapidly that differentiation fails to take place. Another theory is that the cells, for some reason or other, do not age. According to Levine malignancy is due to cytoplasmic alteration rather than chromosomal or gene changes. While the origin of giant cells of cancer is not clear Levine has shown that the foreign-body giant cell and tumor giant cells are not very different except that those that are induced by bacteria are malignant, while those that are activated by non-living granules are harmless, these two types of cells are distinctly separable on a cytological basis.

The cytological study of crown-gall tissue showed no aberrent, no lobulate nuclei, no multipolar spindles and no amitotic divisions. It is noted that binucleate cells and multinucleate cells are common, and that nuclear divisions in crown-gall tissue were of the mitotic type. The striking cytological difference between the crown-gall and cancer cells is the bizarre nuclear division phenonema in the latter,

occurring in the giant tumor cells which were described as uninucleate
and multinucleate cells.

Winge(75) studied the chromosome number in the
crown-gall of the sugar leaf and pointed out that cells in the tumor
tissue had twice the number of chromosomes normally found in the somatic
cells of the leaf. The presence of these increased chromosome numbers
in the tumor cells of the beet was verified (48) and extended to crown-
gall on the tobacco. The results of the various studies made on the
chromosome number in the animal cancer are essentially in accord. It was
pointed out (47) that while the chromosome number in the animal cancer
tissue, including man, occurs generally in multiples of the known haploid
number, there are variations from this.

Crown-gall has at least one vital fundamental
point in common with cancer, and that lies in the fact that crown-gall
is a neoplastic disease consisting of a localized area of proliferating
cells. While the proliferations are limited because the gall ages, the
botanist has the advantage in having an organism which, when injected
into most plants, produces local cell growth and division. Crown-gall
represents essentially a fundamental tissue and, due to the simplicity of
its host, may throw some light on cancer in animals.

Auxins and Bios.

In contrast to Bayliss' definition of hormones
there are such growth promoters as Bios and Vitamin B, necessary in
lower as well as in higher plants. While free movement of these sub-
stances take place within the organism it is not an essential part of
their activity. This is particularly obvious for unicellular organisms.
While microorganisms, cannot, by definition, therefore, have hormones,
the question as to what name be given to their growth promoting substances
remains in doubt. The term vitamin has the definite connotation of a
food factor absorbed from the medium, whereas the growth substances are
in some cases produced by the organisms themselves. For this reason,
Kögl (1935) (34) was led to term Bios a phytohormone. According to
Huxley's nomenclature (1935) (30) we should call such substances local or
intra-cellular activators.

The bios-complex has been mentioned because it
was the first example of a system of interlocking substances each in it-
self a limiting factor and yet the action of each of which is increased
by the presence of the others (Eagles Wood Et al) (19). In 1936 (42)
Kögl and Tonnis isolated from chinese duck eggs a substance they termed
Bios 2 ("Biotin"). Like auxin biotin is active at very high dilutions,
1 part in 4×10^{11} of solution having a detectable effect; however in
terms of the weight of the organism affected its activity is not as high
as auxin.

Root formation in Pisum provides a particularly
good example of a system of limiting factors in higher plants, their
being at least 3 substances known whose action interlocks. These 3 fact-
tors sugar, auxin and biotin are all available in the pure state, and

hence their interlocking action is easily studied. Each factor might

reach a concentration at which it is no longer limiting, and at this

concentration root formation might be increased by adding the next factor,

the increase being proportional to the amount added. The activity of

any one factor alone is zero, the action of sugar alone being ascribable

to the presence of small amounts of auxin in the plant. We have then a

complete parallel to the interaction of Bios 1, 2, and 3 on yeast growth

or to the interaction of enzymes and co-enzymes. Such interlocking systems

of limiting factors we beleive are widely distributed in nature and

probably will be encountered in any process which is sufficiently

analyzed (72v). Grace (23) gives evidence that auxins stimulate yeast and

suggests it might have industrial importance also in this respect. This

seems to conflict with the opinion of Went (1937 "Phytohormones" P. 238)

that "there is no evidence that auxins have any action on fungi."

Rooting Cuttings.

Before roots can grow on a cutting the cambium layer must be stimulated in order that callusing over of the cut surface might take place. In some species a wound hormone or some substance seems to concentrate at the cut surface and increase the activity of the cambium layer to this end. Some species do not seem to take root from their cuttings very readily. Possibly they have been more sensitive to the injury and its ramifications or the wound hormone, if such an agent exists, does not concentrate at that point, or if it does, is not able to stimulate the actifity of the cambium layer. It seems that some factor necessary to stimulate the cambium cell division is lacking, however, because when this is artificially applied in many species, extensive callusing takes place which is soon followed by rooting. Some species bleed their sap a great deal when cut, for this reason they should be cut diametrically in order to keep the wound and loss of sap at a minimum.

Numerous attempts, largely unsuccessful, have been made to correlate root formation or root growth with nutritive factors, especially with the carbohydrate nitrogen ratio (72w). However, it was emphasized by MacCallum (52) that nutrient conditions are not the principal factors governing root formation. Curtis (14) found that permanganate promotes the rooting of cuttings, while inorganic nutrient solutions have no effet. Zimmerman[1730] (76) and Graham[1934] (34) have shown that oxygen is necessary. Treatment with carbon

monoxide ethylene and other unsaturated gases stimulates
root formation, (72x). The most important factors, according
to Graham (24) are:

> (1) time of year at which the cuttings
>   are taken, which differs for diff-
>   erent plants.
>
> (2) temperature relations of the cutting
>
> (3) ample watering without interference
>   withaeration.

They found that if these factors were considered, practically
any plant can be induced to give 90% rooting from stem or
leaf cuttings.

Loeb, in 1917, suggested that root formation
in Bryophyllum is controlled by a special root forming sub-
stance or hormone. About this substance he made some state-
ments which in light of our present knowledge seem quite re-
markable. He said: "In Bryophyllum the hypothetical geotropic
hormone is associated (or identical) with the root forming
hormone. Further these inhibitory substances may be identical
with or may accompany the root-forming hormones."

In many cases it has been shown that the
presence of buds and leaves promotes root formation. Removal
of buds and leaves stops root formation almost completely,
especially in the species without root germs (root nodule
bacteria). Van der Lek assumes that the developing bud
forms one or more hormones which are transported downward
through the phloem. Van der Lek also found that in dormant
populus cuttings taken in December or in January, the buds
no longer promote root development or even slightly inhibit
it, but in the course of the next two months their favorable

influence returns. From a great number of experiments, workers to-day believe that root formation in most cases is due to a thermostable hormone, not itself a nutrient and produced by leaves in the light. It is furthermore stored up in cotyledons and buds, and its transport is basipetally polar.

In 1935 Kogl tried auxin b and later indole-3-acetic acid and found them to stimulate rooting. Many hetero-auxins derivatives were them tried and they were found to be successful in promoting roots on cut ends. There is good reason to believe that the nodules produced by root nodule bacteria are a result of the stimulation of the cambium by indole-3-acetic acid produced by those organisms. It was these preliminary theories and experimental results which led to the appearing on the market of several very commerciable synthetic substances encouraging rooting of cuttings. Examples of two of these under their trade names are Hormadin/A and Auxilene. It is believed they are all derivatives of indole-3-acetic acid.

Other Limiting Factors Necessary for the Production of Roots.

Carbohydrate applied at the base is necessary for root production by an etiolated cutting (74y). The kind of sugar used seems to be very important. Sucrose and fructose give larger numbers of roots than dextrose under comparable conditions. These sugars also affect the length of the root produced in the same manner.

There are other substances which are like the

sugars in that they must be applied to cuttings from the base, but are unlike the sugars in that the amounts of them necessary to influence root formation are extremely small. In this case I refer to bios. Its function as a limiting factor has been mentioned previously in this discussion. To produce a visible root at least three processes must take place in succession: redifferentiation of pericycle cells into root initials, formation of root primordium by these initial cells, and the outgrowth of the root primordium. From a standpoint of morphogenetics, the first two processes are the most important, physiologically speaking they can be regarded as one process. There several reasons for thinking that it is this process which is influenced by the factors discussed above. First the length of the roots is not materially influenced by the treatment, except by sugar(Went) (72z). Secondly examination of pea stems has not revealed any appreciable number of root primordia. Thirdly we know that the direct effect of auxin on roots is to inhibit their growth in length. Furthermore, the total length of roots formed per cutting is more or less constant, so that the more roots ar formed, the shorter they are. This indicates that the outgrowth of the roots is not influenced by the auxin treatment but by an internal factor which may become distributed over a large number of root primordia.

There is, however, apparently another factor of quite different type which apparently also takes part in the first stages of root formation. If a pea cutting is divided into a number of sections and each is placed in a

sugar solution and treated with high concentrations of auxin
(in lanoline paste form) then the sum of the number of
root primordia formed is about the same as if the intact cut-
ting were so treated. The total number of primordia is thus
nearly constant and must therefore be determined by an in-
ternal factor other than the auxin which factor only becomes
limiting when auxin is in excess. The distribution of this
factor inside the plant can be determined from the dis-
tribution of the primordia on the dissected cuttings; the bulk
of the primordia are on those sections some distance from the
apex which therefore contained the most of this factor. How-
ever when auxin was applied to the intact cutting, 30 primordia
were formed at the top, but when applied to the uppermost, one-
eighth of a cutting only about 7 were formed at the top. The
auxin may therefore mobilize some of the other factor from
the lower parts of the cutting. This suggests that an import-
ant function of the auxin is to control the movement of this
factor.

Except when applied in very high concentrations
(.00002 grams per cc.) auxin must be applied at the apex to
induce root formation at the base. The commercial preparations
and indene-acetic and cumaryl acetic acids whose transport
from apex to base is limited give better results of course
when applied at the base.

It will be seen from all this discussion that
in the complex process of root formation several factors are
involved. These include auxin, carbohydrates, a group of

other substances such as biotin, and the internal factor discussed above.
Very little is known of this factor save that auxin seems to control its
distribution. Much further study will be needed to elucidate their
interactions. (72a)

Commercial Applications.

Cooper, using hetroauxin in lanoline in 1935
probably did the first work on rooting of cuttings of commercially
important plants. In 1936 Hitchcock and Zimmerman used heteroauxin in
high concentration and made up a large list of plants commercially feasible
in this respect. This list has been extended by the private reports of
a number of horticulturists. In general the highest non-toxic concentrat-
ion of indole-acetic acid, dissolved in water will give the best results.
This concentration varies for different plants and is lowest for green
cuttings. A treatment with 0.2 mg. per cc. for 12 to 24 hours can be
recommended, but before large-scale applications are made, each species
toxic limit should be found out by experiment. Indole butyric and nap-
thalene-acetic acids are also effective. For treatment by the lanoline
method a concentration of about 1 mg. of indole -3- acetic acid per
gram of lanoline is satisfactory. With many horticullurists in Holland
and Scotland the practice of putting a germinating wheat seed into the
apical split end of the cutting has been practiced. Moreover in non-
deciduous plants, leafy cuttings are generally used, probably because no
auxin is stored in their stems. In deciduous plants leafless cuttings
are used or the leaves are half clipped because of the difficulty with
water supply to the leaf, but here the bud acts as an oxygen supply.
For example Hitchcock and Zimmerman found that leafless cuttings of the
deciduous Ilex verticillata would root but those of the evergreen varieties

failed to root.

The success of layering probably depends on the retarding influence on auxin transport exerted by high humidities, together with the geothopic accumulation of auxin on the lower side of the stem. Rooting takes place usually at nodes probably because there the transport of auxin is interfered with. For this reason the cuttings are made at nodes in most cases. The practice of tying tight wires around branches or cutting cortex during growth is probably another example of early horticultural practices designed wittingly or otherwise, to affect the production and movement of auxin.

The optimum time of year to take cuttings varies among different plants (24) and depends upon a number af factors such as water supply and ease of wilting, auxin production storage, and destruction. (7124) Many cuttings fail to root when treated with auxin. This is possibly due to many causes, one of which might be that, not auxin but one of the other necessary materials mentioned in our interlocking system of limiting factors is missing. Another cause might be the loss of the applied auxin or other factors by exudation from the cut surface; cuttings which root with difficulty are found to be those from which much exudation takes place. Lastly the inactivation of auxin at the cut surface, by oxidative enzymes set free in wounding, might take place.

Suggested Improvements on Treatment of Cuttings.

Treatment of cuttings by a dusting treatment showed a superiority over the solution treatment. In many cases it was found more effective and in cases in which it was no better in its results than the solution treatment. Its simplicity and convienience recommend it for

practice. The hormone is incorporated in some absorbent dust. For example 2.5 - 25 parts of hormone per million of talc dust. Bunches of cuttings are dipped into the dust and the excess is shaken off. Further experiments may prove that the solution treatment might be more suitable for certain species or conditions while the dusting treatment would be more adaptable to others.

Immersing the base of partly wilted lettuce plants and cut flowers in appropriate concentrations causes these to regain their turgor and freshness in a remarkable way. The life of certain cut flowers may be prolonged. Indole -3- acetic acid, its butyric and propionic homologues; naphthyl-acetic acid and their salts and mixtures gave similar results.

The Nature of the Auxin Action.

Auxins bring about a number of different responses in plants and seem to influence a large number of processes, both normal and pathological. Some of these responces are, specifically: growth by cell elongation; the formation of roots; the inhibition of buds; the activation of the cambium, inhibition of root growth, certain growth phenomena involving cell enlargement and cell division together, a stimulating action on yeast cells and possibly some unknown effect on animal tissue. The literaturs shows that a number of different substances can bring about the same growth response. Went maintains that any one of the active substances can bring about all these different responses. (7azc) Its activity may sometimes be concealed, as in those substances which cause no Avena curvature, but it may be demonstrated by special experiments. Many of the apparently different effects may be traced to different types of cell enlargement, but others, such as root formation are more complex.

All this raises the question of the mechanism of these affects. There are two possibilities which may be considered (1.) the auxins bring about some mast-reaction within the cell, the results of which will be determined by the presence and amount of other factors ("condition of the cell"), and by the conditions of the experiment; or (2.) the auxins are stimulating substances possibly acting on the enzymes in some way to set free the energy stored up in living protoplasm. According to Fitting (1936) (2/) "stimulus-substances" are those substances which exert their physiological action through the intervention of the living substance, ie. whose first point of attack is the living plasma. In the typical physiological action then should appear all the characteristics of stimulations, eg. latent time, presentation time, threshold concentration,

exitation of the protoplasm, anti-reaction and recovery, etc. (Bayliss)

(3). However Went showed that the responces of plants to auxin are not typical stimulus responses and he also shows that the relation between auxins and growth has nothing in common with stimulation (Went) (72z4) inasmuch as a given amount of auxin produces a given amount of growth; the curve of auxin applied against growth passes directly through the origin, therefore there is no evidence for a threshold in the response of young Avena coleoptiles to auxin. The stoichiometric relations of a number of substances, in the pea test show thata number of substances approach the same value per molecule. This together with the linear proportionality just mentioned, means that the auxins enter into a definite stoichiometric reaction with some constituent of the cell. The concept of stimulus and all that it implies, has therefore no useful bearing on auxin problems.

There remains the other possibility previously mentioned, that af a master-reaction. According to this view auxin acts by taking part in some reaction in the cell, from which a chain of reactions lead to the observed response. The type of response then depends on the other factors both internal and external, influencing the reaction chain (Thimman 1935) (68). As previously mentioned growth is controlled not only by auxin but also by another factor or group of factors; the most rapidly growing zone is that in which both are present in optional concentrations. The auxin in the extreme tip of the coleoptile cannot cause growth because the others factors are limiting. The auxin at the extreme base of the coleoptile cannot cause growth because the cells can no longer respond to it. Root formation furnishes a still more striking example. Here, the formation of roots is dependent upon the cooperation of a number of factors. The concentration of auxin determines not only the number of roots formed,

but also the cells which will form them. In general the response in organ formation is localized. To explain this localization of the action Went (1936) (7/) has suggested that in addition to its master-reaction effect, auxin acts by affecting the transport of the other factors, so that they become accumulated at the point of highest auxin concentration. However this may be, when the influence of the other factors is taken into account, the apparently mysterious action of the auxins in bringing about so many responses no longer seems so obscure. It is true that the nature of the fundamental master-reaction is not known, but we believe at the present rate of progress in this field the solution of this perplexing problem will soon be complete.

Another possible field of attack might be the affect of auxin on the permeability of the cell wall directly or on some other substance which affects the permeability of the cell wall. If it could be shown that auxins make the wall more permeable it might be concluded they improve the conditions for the transportation of substances necessary to growth, and thus in this way play their part in stimulating growth. It seems that this direction of attack would be more likely to account for the reason so many substances bring about the physiological effects which mark auxins. It has been known for some time that plant tissues contain ethylene (13). It was previously stated that ethylene will not bring about auxin effects without the presence of auxins. By increasing the ethylene supply in our seed pre-treatment we recorded a marked stimulation in growth. Is it possible that previous to this ethylene was one of the limiting factors. Heyn et al in 1936 (29) has shown that auxin increases the plasticity of the cell wall. Just what this relation between this and permeability is might be hard to say. From calculations of moleculaar concentrations it has been shown (Went, 1937) (72ze) that

there is not nearly enough auxin to form even a monomolecular film over

the surface of the new cell wall which it produces by which it might

directly affect permeability; thus auxin acts in some indirect way. It

was mentioned earlier (23) that wilting cut flowers are stimulated by

putting auxin in the water in which their cut ends are immersed. It is

very likely that this is an indication fo auxin's ability to affect the

permeability of cell walls.

CONCLUSION

1. Self-inactivation does not take place as completely when the auxin a is in a relatively impure dilute state. Just which factor was more important in preventing self-inactivation, dilution, or some substance present in the impurities is unknown. To raise the pH and convert the auxin a to its sodium salt and keep the solution in a dilute condition would possibly serve as a means of retaining the active factor over a long period of time.

2. There are many substances natural and synthetic which bring about cell elongation to varying degrees and are therefore termed auxins. It is suggested the word auxins be reserved for substances of this nature that occur naturally in the plant kingdom.

3. Many ways have been devised for determining the ability with which a substance promotes cell elongation, however the Went test technique of coleoptile curvature is still the most popular and generally used inasmuch as such affects as residual growth, zero reading, etc. are obviated. A variation of the Went test technique is suggested and its advantages enumerated.

4. The auxins are apparently not germination hormones although they are found to affect the subsequent development of certain organs as well as the yield from reproductive parts. There is evidence that auxin and ethylene may augment one another.

5. A pre-cursor, possibly the auxin ester, is beleived to be provided in the endosperm. During early growth this is assumed to travel towards the apex where it is converted into active auxin which then migrates basally to bring about growth in terms of cell elongation. Just when or how it

plays its part in the initiation and subsequent development of roots is still a question. When the auxin pre-cursor is used up in the endosperm then its production or that of pure auxin takes place in the apex in the presence of light. A germination hormone is beleived to exist however and it has been called "blastanin". It is beleived that its migration from the endosperm to the embryo takes place during vernalization and is there in a position to affect subsequent growth. Its affect seems to be modified by the presence of ascorbic acid.

6. According to Grace (1937) auxins promote the activity of yeast. This in contrary to opinions prior to her work.

7. Evidence is presented to show that auxins will bring about tumors not only on plant tissues but on animal tissue. The similarities and differences of the two are discussed. Parallels and differences are also drawn between plant and animal hormones.

8. Some work was done on the rooting of cuttings from a number of species.

9. Just why auxin promotes stem elongation and inhibits that of roots is unknown. W. J. Robbins, 1937, (77) however finds that the extensibility of cell walls from stems are increased but that of roots are decreased when treated with B-indole-acetic. This may partially explain why the hormone increases growth of stems but inhibits that of roots. The stem walls are apparently physically and chemically different from root walls.

10. To date the beleif is that auxin brings about some master reaction rather than recognizing them as stimulating substances. Auxin is one or a group of limiting factors working together to bring about the development of the mature plant.

BIBLIOGRAPHY

1. Avery, jr., G. S. and R. R. Burkholder, 1936. Polarized growth and cell studies on the Avena coleoptile. Bull. Torrey Bot. Club 63: 1 - 15.

2. Barton, U., 1930. Germination in conifers and effect of Temperatures. Boyce Thompson Institute. Page 331.

3. Bayliss, W. M., 1927. Principals of general physiology.

4. Berridge, E. M., 1930. Studies in bacteriosis. Ann. Appl. Biol. 17: 289 - 283.

5. Bonner, J., 1933. The action of the plant growth hormone. J. gen. physiol. 17: 63 - 76.

6. Boysen Jensen, P., 1932. Uber die Bilding und biologische Bedeutung des Wachstumsregulators bei Aspergillus niger. Biochem. Zeits. 250: 270 - 280.

7. Boysen Jensen, Avery and Burkholder, 1936. Growth hormones in plants.   a - page 52.   b - page 21.   c - page 16.   d - page 67.

8. Buy, H. G.,du, and E. Nuernbergk, 1932. Phototropismus und Wachstum der Pflanzen. Ergeb. Biol. 9: 358 - 544.

9. Cholodny, N. 1935. Investigations on the growth hormone of plants. Herbage Review, 3: 210 - 214.

10. Cholodny, N., 1936. Hormonization of grains. Herbage Abstracts, Vol. 7, No. 3, September.

11. Cholodny, N., 1937. On the theory of yarovization. Herbage Abstracts Vol. 7, No. 3, September.

12. Clarke, W. G., 1937. Ascorbic acid in the Avena coleoptile. Bot. Gazette, September 1937.

13. Crocker, W., Hitchcock and Zimmerman, 1935. Similarities in the effects of ethylene and the plant auxins. Contrib. Boyce Thompson Inst. 7: 231 - 248.

14. Curtis, O. F., 1918. Stimulation of root growth in cuttings by treatment with chemical compounds. Cornell Univ. Agr. Expt. Sta. Mem. 14.

15. Darwin, C., 1880. The power of movement in plants. London.

16. Davis, W., 1937. Effect of hormones on germination of seeds. Annals of Bot. London. 1: 239 - 351.

17. Davis, W., Atkinson and Hudson, 1937. Annals of Bot. Vol.1., No. 2, page 349.

18. Denny, F. E., 1934. Cormel treatment with ethylene chlorhydrin. Contrib. Boyce Thompson Instit. Vol. 6.

19. Eagles, B. A., and A. Wood, 1936. Can. Jour. Research. B 14: 151-154

20. Feiser, L. F., 1936. The chemistry of natural products related to phenanthrene. A. C. S. Monograph, New York.

21. Fitting, H., 1936. Die Hormone als physiologische Reizstaffe. Biol. Zentralbl. 56: 69 - 86.

22. Goodwin, R. H., 1937. The role of auxin in leaf development, in Solidago species. Am. Jour. of Bot. 24: 43 - 51.

23. Grace, U., 1937. Treating seeds and cuttings with hormones. Can. Journ. of Research. Vol. 15. No. 11. 5 - 38. November.

24. Graham, R. J., 1934. The work of Laurence B. Stewart. Trans. Bot. Soc. Edin. 31: 450 - 459.

25. Gregory, F. G., 1928. Studies in the energy relations of plants.
    Ann. Bot. 42: 469 - 507.

26. Gustafsen, F. G., 1937. Parthenocarpy induced by pollen extracts.
    Amer. Jour. of Bot. 24: page 102.

27. Haagen Smit, A. J. and F. W. Went, 1935. A physiological analysis
    of the growth substance. Proc. Kon. Akad. Wetensch. Amster-
    dam. 38: 852 - 857.

28. Handley, W. S., 1922. Cancer of the breast and its treatment.
    Second Edit.

29. Heyn, A. N., and J. van Overbeek, 1931. Weiteres Verssuchmaterial
    sur plastischen und elastischen Dehnbarkeit der Zellmembran.
    Proc. Kon. Akad. Wetensch. Amsterdam. 34: 1190 - 1195.

30. Huxley, J. S., 1935. Chemical regulation and the hormone concept.
    Biol. Review. 10: 427 - 441.

31. Jensen, C. O., 1910. Von echten Geschwulsten bei Pflanzen. Rap-
    port de la deuzieme internat. conference pour l'etude de cancer.
    Paris. 243 - 254.

32. Jost, L., and E. Reiss, 1936. Zur Physiologie der Wuchsstoffe.
    Einfluss des Heteroauxins auf Langen-und Dichenwachstum.
    Z. f. Bot. 30: 335 - 376.

33. Kögl, F., 1933. Phytohormone der Zellstreckung. Z. physiol. Chem.
    (Translation by Department of Botany, U. B. C.)
    a - page 4. b - page 6. c - pages 7 & 8. d - page 3.
    e - page 18. f - page 17. g - page 12.

34. Kögl, F., 1935. Uber Wuchsstoff der Auxin-und der Bios-Gruppe.
    Ber. der chem. Ges. 68: 16 - 28.

35. Kögl, F. 1936. On the self-inactivation of auxin a. Translation
    available. (J. H. W. Willard). Hoppe Zeylers Zeitscrift fur
    Physiological Chemie. Vol. 6. Page 266.

36. Kögl, F., and H. Erxleben, 1934. Uber die Konstitution der Auxine
    a und b. X. Mitteilung uber pflanzliche Wachstumsstoffe.
    Z. physiol. Chem. 227: 51 - 73.

37. Kögl, F., and H. Erxleben, 1935. Synthese der "Auxin-glutarsaure"
    ind einiger Isomer. XV. Mitteilung. Z. physiol. Chem. 235:
    181 - 200.

38. Kögl, F., and A. J. Haagen Smit and Erxleben, 1933. Uber ein Phyto-
    hormon der Zellstreckung. Zur Chemie des Krystallisierten
    Auxins. V. Mitteilung. Z. physiol. Chem. 216: 31 - 44.

39. Kögl, F., and A. J. Haagen Smit and H. Erxleben, 1934. Uber die
    Isolierung der Auxine a und b aus pflanzlichen Materialen.
    IX. Z physiol. Chem. 225: 215 - 229.

40. Kögl, F., and A. J. Haagen Smit and H. Erxleben, 1934 a. Uber den
    Einfluss der Auxine auf das Wurzelwachstum und uber die
    chemische Natur des Auxins der Graskoleoptilen. Xll.
    Mitteilung. Z. physiol. Chem. 228: 104 - 112.

41. Kögl, F., A. J. Haagen Smit and B. Tonnis, 1933. Uber das Vorkom-
    men von Auxinen und von Wachstumsstoffen der"Bios-Gruppe"
    in Carcinomen. Vlll. Z. physio.. Chem. 220: 162 - 172.

42. Kögl, F and B. Tonnis, 1936. Uber das Bios-Problem. Darstellung
    von Krystallisiertem Biotin aus Eigelb. Z. physiol. Chem.
    242: 43 - 73. Translation available (J.H.W.Willard).

43. Kögl, F, and D. Kostermans, 1935. Uber die Konstitutions Spezifitat
    des Heteroauxins. XVl Mitteilung. Z. physiol. Chem. 235:
    201 - 216.

44. Koningsberger, c.,1936. De auto-inactiveering der Auxinen. Diss Utrecht,

45. Laan, P. A. van der, 1934. Der influss von Aethylen auf die Wuchsstoffbildung bei Avena und Vicia. Rec. trav. bot. neerl. 31: 691-742.

46. Laibach, F., and Kornmann, P., 1933. Zur Frage der wuchsstofftransportes in der Haferkoleoptile.Planta 21: 396-418.

47. Levin, I., and Levine, M., 1920. Malignancy of the Crown-gall and its analogy to animal cancer. Jour. of Cancer Res. 5: 243-260.

48. Levine, M., 1930. The chromasomi number in cancer tissue of man, of rodent, of bird, and in crown-gall tissue of plants. Jour. of Cancer Res. 14: 400-425.

49. Levine, M., 1931. Studies in the cytology of cancer. Am. Jour. of Cancer. 15: 144-211.

50. Levine, M., 1936. Plant Tumors and their relation to cancer. Bot. Rev. vol. 2 No. 9, a- 443, b- 447.

51. Lilly, U., and L. H. Leonian, 1937. Is heteroauxin a growth-promoting substance. American Jour. of Botony.

52. McCallum, W. B., 1905. Regeneration in Plants. Bot. Gazette 40: 97-120.

53. Maschmann, E., 1933. Das Vorkommen von Wuchsstoff in tierischem und pflanzlichem Material. Naturwiss 21: 517.

54. Navez, A. E., and B. Kropp, 1934. The growth-promoting action of crustacaan eye-stalk extract. Biol. Bull. 67: 250-258.

55. Nielsen, N., 1937. Growth substance content of seeds of varying ages. Herbage Abstracts. Vol. 7 No. 4: 316.

56. Robinson, T. W., 1937. Auxin in the chick embryo. J. Cell. comp. Physiol. 9: 241-260.

57. Sachs, J., 1880. Stoff und Form der Pflanzenorgane 1.and 11. Arb. Bot. Inst. Wurzburg 2: 452-488 and 689-718.

58. Scheer, B. A., 1937. Straight growth method of testing auxins. Am. J. of Bot. No. 9, Vol 24, P. 559.

59. Schoeller, W., and H. Goebel, 1935. Die Einwirkung astrongener Substanzen auf Pflanzen lv. Biochem. Z. 278: 298-311.

60. Schwantje, E. A., 1937, University of B. C.

61. Silberschmitt, K., 1928. Untersuchungen uber die Abhangigkeit des phlanzlichen Wachstumverlaufes und der erreichten Endlange von konstanten Temperaturgarden. Bibliotheca Botanica 97, Stuttgart.

62. Skoog, F., 1937. A deseeded Avena test method for small amounts of auxin and auxin precursors. J. gen. Physiol. 20: 311-334.

63. Smith, E. F., 1917. Mechinism of tumor growth in crown-gall. Jour. Agr. Res. 8: 165-186.

64. Snow, R., 1935. Activation of Cambial growth by pure hormones. Nature 135: 876.

65. Solocalu, T., 1937. The action of indole -3- acetic on the germination of grains. Biol. Abst. 18215, Nov. 1937.

66. Tetley, U. and J. H. Priestly, 1927. The histology of the coleoptile in relation to its phototropic response. New Phytol. 26: 171-186.

67. Thimann, K. V., 1935. Growth substances in plants. Ann. Rev. Biochem. 4: 545-568.

68. Thimann, K. V., 1935 b. On analyses of the activity of two growth-promoting substances on plant tissue. Proc. Kon. Akad. Wetensch. Amsterdam 38: 896-912.

69. Thimann, K. V., and J. Bonner, 1932. Studies on the growth hormone of plants. 11. The entry of growth substance into the plant. Proc. Nat. Acd. Sc. 18: 692-701.

70. Weij, H. G., van der, 1931. Die quantitative arbeitsmethod mit Wuchsstoff. Proc. Kon. Akad. Wetensch. Amsterdam, 34: 875-892.

71. Went, F. W., 1936. Allgemeine Betrachtungen uber das Auxin-Problem Biol. Zentralbl. 56: 449-463.

72. Went, F. W., and K. V. Thimann, 1937. Phytohormones.
    a - chapter 3 c, b - page 7, c - page 22, d - page 23, e - page 28, f - page 45, g - page 34, h - chapter 3, i - page 140, j - page 54, k - page 115, l - page 116, m - page 114, n - page 116, o - page 66, p - page 184, q - page 71, r - page 233, s - page 3, t - page 234, u - page 198, v - page 238, w - page 183, x - page 184, y - page 194, z - page 199, za - page 202, zb - page 206, zc - page 244, zd - page 246, ze - page 128, zf-p.21,

73. Whyte, R. O., and P. S. Hudson, 1933. Lyssenco's method for pre-treatment of seeds. Imperial Bureau of Plant Genetics, Gr. Britain. March. Bull. No. 9.

74. Willard, J. H. W., 1937. Paper to B. C. Academy of Science on Plant Hormones, January.

75. Winge, O., 1927. Zytologische Untersuchungen uber die Natur naligner Tumpren. Crown-gall der Zuckerrube. Zeits Zellforsch Mikr. Anat. 6: 397-423.

76. Zimmerman, P. W., 1930. Oxygen requirements for root growth of cuttings in water. Am. Jour. of Bot. 17: 842-861.

ADDENDA

77. Robbins, W. J., 1937. Effect of indole-3-acetic on cell walls of stem and root. Amer. Jour. of Bot., 24, March.