THE RELATION OF AUXIN TO THE EXTENSION GROWTH OF THE STEM IN CEREALS

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S U M M A R Y

1. A method of plant auxin assay based on the quantitative relationship of auxin concentration to the root growth of rye seedlings has been developed.

2. The effect of carbohydrate on the production of auxin in excised embryos of Petkus winter rye has been studied by this method.

3. The distribution of auxin in the coleoptile, Coleoptile/node, leaf and root of excised embryos of different ages has been determined.

4. Low temperature (2 °C) did not influence the production of auxin in embryos, whereas at high temperature (35 °C) auxin disappeared from the embryo.

5. Oxygen was essential for both growth and auxin production in excised embryos.

6. Auxin content of the apex, developing ear and extending stem of Petkus winter rye in relation to vernalisation and day length has been studied. There was no appreciable change of auxin content in the apex either before or immediately after floral initiation. Irrespective of treatment and time from planting, the amount of auxin in the plant is approximately the same at the same stage of floral development.

7. The auxin content of plants transferred (A) from long days to short days and (B) from short days to long days was studied. Short days were unfavourable for stem elongation in rye, but if the plant could attain a certain critical stage of floral development before transfer from long days, the further extension of the stem was possible under short days.

8. The auxin content of the nodes and internodes of the stem was found to bear a clear relationship to extension growth of the internodes.

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INTRODUCTION

Since the pioneer experiments of Sachs (1880, 1882) which indicated that plants produced organ forming substances a mass of information has accumulated concerning substances which profoundly influence the growth of plants; these are known under the general term auxins or growth substances.

Auxin was observed in the tip of the coleoptiles of Avena as early as 1910. Boysen - Jensen (1910, 1913) observed that the phototropic stimulus which was generated in the tip of the coleoptile can cross a discontinuity and suggested that this stimulus was of a "material nature". Paál (1919) first obtained a curvature in the coleoptile of Coix by replacing its cut tip eccentrically, and suggested that a substance was formed and internally secreted which accelerated extension of the cells. Went (1926, 1928) was able to transfer this substance by diffusion from coleoptile tips to agar blocks. The blocks in turn produced curvature in decapitated Avena coleoptiles. This was the basis of his standard Avena coleoptile technique.

Since then numerous workers have demonstrated the presence of auxin in various parts of the plant and studied its relative amount, its activity and its chemical nature.

Thimann and Skoog (1934), Avery (1935) have detected the high concentration of auxin in herbaceous plants e.g., Vicia, Nicotiana. Zimmermann (1936) has studied hormone concentration in developing shoots of Acer pseudoplatanus, Fraxinus excelsior, and several other woody species. Laibach and Meyer (1935) determined by alcoholic extraction the concentration of auxin at different periods in the life cycle of Zea mays and Helianthus. Auxin has also been demonstrated in several other woody stems as in plum and apple shoots (Hatcher and Garner 1948, Hatcher 1948), in the seedling axis of several herbaceous dicotyledons (Van Overbeek 1933) and in nodes and internodes of grasses (Van Overbeek 1938, 1943, 1944, 1945).

In general, the growth of the apex and elongation of the shoot below it are known to be closely connected with the production of auxin by the apex (Thimann and Skoog 1934). The details of the relationship are by no means clear. In herbaceous plants the terminal bud is the main source of diffusible auxin and lateral buds which are not developing yield practically none (Thimann and Skoog 1934). Zimmermann (1936), Avery et al (1937), Hatcher (1948), Hatcher and Garner (1948) have shown that the period during which diffusible auxin can be obtained in large quantities from apices, internodes and nodes of plants is quite short.

During spring growth within a period of a few weeks the auxin yield rises rapidly to a maximum and falls back almost to zero. Zimmermann (1936) emphasizes a parallel between growth intensity and growth hormone concentration in shoots and concludes "where there is auxin there is growth." Avery et al (1937), however, have found in Aesculus and in Malus that during the spring growth concentration of diffusible auxin in the stem rapidly increases to its peak value about 3 weeks prior to any visible swelling of the buds, then with the extension growth of the shoot the concentration of auxin gradually falls to zero. In the inflorescence bearing shoots, however, this decline is not so marked; as the young fruit begins to grow, the auxin content of the fruit-bearing shoots is sustained at a higher level than is that of the vegetative shoots. The concentration of auxin in the developing inflorescence of Aesculus Hippocastanum is found to increase gradually and attained a high level as the fruit start to develop.

A series of studies of native auxin of grasses has been done by Van Overbeek (1938, 1943, 1944, 1945). He has elaborated a useful technique for extraction of free auxin from grass internodes at low temperature using ether as a solvent. There appears to be a considerable difference in free auxin content between nodes and internodes. Nodes of grasses contain much more auxin than internodes; again under the influence of geotropism the free auxin of nodes increases to more than twice its initial amount while auxin level of

internodes remains unaltered. By a thorough investigation (Van Overbeek 1945) the diffusible auxin of grass internodes has been proved to be a thermostable substance of the type of indoleacetic acid.

Hatcher (1945) has made a comprehensive study of auxin production in cereal grain during the period of development and embryo formation. In both winter and spring varieties of rye auxin is rapidly produced and reaches a maximum some 7½ - 8 weeks after anthesis. This maximum coincides with the stage of complete differentiation of the embryo and is followed by a rapid fall in auxin level up to the complete maturity of the grain. It is suggested that the apparent disappearance of the auxin is associated with desiccation of the grain.

Auxin in relation to vernalisation and floral initiation.

There is some difference of opinion regarding the relation of auxin to vernalisation. Cholodny (1936, 1939) put forward the hypothesis that during the prolonged contact between the embryo and the endosperm, the embryo absorbs auxin that is present in the endosperm. This would lead to an increase in the concentration of auxin in the cells of the embryo accelerating the progress of the meristematic cells of the young plant through the earlier phase of development with the result that the later stages are shortened. Gregory and Purvis (1936, 1938) have shown that excised embryos entirely free from endosperm tissue can be vernalised. Thus it is clear that auxin from the endosperm is not an essential factor in the process. Sircar and Das (1951) have demonstrated a high concentration of auxin in vernalised rice grain, and this concentration has been shown to decrease with the growth of embryo at favourable temperature. Low temperature stimulation in rice, however, does not lead to the acceleration of flowering but rather to increased vegetative growth as indicated by tillering and height (Sircar and Pariza 1949), therefore, no relation of high auxin concentration in the grain to the acceleration of flowering is indicated.

The relation of auxin to the initiation of flowering has been stressed by a number of workers. Assaying auxin in a variety of species Cailahjan (1947) and Cailahjan and Zdanova (1938) concluded that plants grown under short photoperiods possess less diffusible auxin than plants grown under long photoperiods regardless of the photoperiodic behaviour of the plant. Galston (1947) failed to find any consistent effect of photoperiod on the ether-extractable auxin derived from apical buds or leaves of soybeans. Hamner and Bonner (1938) found that application of indoleacetic acid in lanolin reduced the intensity of the flowering response in Xanthium. Galston (1947) also obtained similar quantitative reduction in flowering response of soybeans growing in short days by spraying with auxins (Indoleacetic acid and Napthaleneacetic acid). Bonner and Thurlow (1949), also Bonner (1949) have observed that flower formation of short day plants can be suppressed by treating the plants with auxin or synthetic growth regulating substances and can be induced under non inductive conditions by treatment with auxin antagonists (including 2, 4 - dichloranisole (D C A) and 2, 3, 5 - triiodobenzoic acid) or other agents which ~~destroy~~ interfere auxins. On the other hand Leopold and Thimann (1949) have reported that injection of Indoleacetic acid in low concentration through leaves of Barley increases the number of floral primordia though there is no evidence that flower initiation was accelerated.

These observations led them to conclude that low concentrations of auxin may promote flowering while higher concentrations inhibit flowering.

Little is known of methods of lowering the level of natural auxin in the plant. Hitchcock and Zimmermann (1951) have examined critically the observations of Galston (1947), Bonner (1949) and of Bonner and Thurlow (1949) mentioned above regarding the antiauxin activity of T I B A as it affects floral initiation in soybean and Xanthium. They state that regardless of whether T I B A causes a change in level of extractable substances that are active in the Avena test, this could not in itself constitute a proof that the said active substances would normally play any part in flowering of the plant.

In the present study it appeared important, therefore, to undertake a direct study of the native auxin of the plant before and after floral initiation which may elucidate the actual changes of native auxin in the plant during this period. It is well known that the growth of the cereals takes place in two phases (a) at first a continuous production of leaves and laterals on a compressed main axis, (b) and after the initiation of flowers at the stem apex a rapid extension growth of stem and development of the reproductive organs and accessories during which the extension of tiller buds is largely suppressed.

Wittwer (1943) drew attention to the relation between reduction division and the rapid production of auxin after fertilization in Zea mays. Hatcher (1945) showed in the case of winter rye that the auxin content in the carpel rapidly increased after fertilization and subsequently fell during the ripening of the grain. It should be noted, however, fertilization in rye plants takes place after ear emergence at which time stem elongation is nearly completed. In rye stem elongation usually begins after initiation of spikelet primordia; it, therefore, becomes a matter of importance to discover whether any change of auxin content of the growing point of the plant occurs either preceding or following the floral initiation. The time of initiation of winter cereals can be controlled by environmental factors such as temperature or day length. In this work the time of initiation has been controlled by such means and the auxin content of the developing apex and extending internodes in relation to these treatments has been determined.

The high auxin content of the endosperm of rye, rice and other cereals has led to the suggestion that auxin supplied by the endosperm is needed by the growing embryo. No previous work has been carried out to prove this fact directly. Preliminary observations by Sircar and Das (1951) showed that the total auxin content in the endosperm of rice released by alkalihydrolysis extraction (at pH 9.5) fell during the course of development of young seedlings.

As, however, the embryo remained in contact with the grain and was not tested separately, it is not known whether the auxin released from the endosperm eventually accumulated in the embryo. In the course of the present work the capacity for auxin production of embryos separated from the grain has been studied in some detail. It has been shown that isolated embryos are capable of synthesising auxin without intervention of the auxin precursor derived from the endosperm. The production of auxin in isolated embryos has been studied in relation to the carbohydrate and oxygen supply and to the temperature.

Chemical nature of auxin found in plant

Recently the chemical nature of auxin in plants has received considerable attention (Veldstra 1953), especially by chromatographic methods (Bennet-clark et al 1952, Luckwill 1952, Terpstra 1953, Audus et al 1953). In general two auxin systems have been recognised in plants, one which includes the auxin a and b, the other is the indoleacetic acid system. Haagen-Smit et al (1942) have isolated indoleacetic acid from the maize grain and this has been repeated by Berger and Avery (1944). The formation of indoleacetic acid from the isolated plant protein of the leaves of higher plants has been demonstrated by Gordon and Wildman (1943, 1946). Haagen-Smit et al (1942) have isolated indoleacetic acid from corn meal and indicated that most of the auxin in the hydrolized meal is indoleacetic acid. Van Overbeek (1945) has identified the auxin extracted from grass stems by ether at low temperature with indoleacetic acid. By chromatographic test Terpstra (1953) has shown that water and ether extract of coleoptile tips of Avena likely consists of indoleacetic acid only. Indoleacetic acid with one other substance hawe been demonstrated in etiolated sunflower seedlings by Bennet-Clark et al (1952) with chromatographic test. It is quite possible that indoleacetic acid is widely distributed in higher plants ~~perhaps~~ more widely than auxin a and b (Thimann 1948).

M A T E R I A L AND M E T H O D S

Material

The present work was carried out with Petkus winter rye (supplied by Pure Seed Co., Ltd., Linton). Plants were grown in sand culture with controlled nutrition (see table 2); samples were taken from them at intervals for auxin estimation. Petkus winter rye was also used for culture as test plants by means of which auxin was assayed.

Methods of Assay

The inhibition of root growth in rye seedlings was found to bear quantitative relationship with the concentration of auxin presented to them in the medium on which they were cultured. This relationship has been used for the assay of auxin in this work. The details of the test method were as follows:- Seeds of Petkus winter rye were dusted with commercial "Spergon" (to prevent fungal contamination) and were soaked in sterilized distilled water for 2 hours at 20°C. They were then allowed to germinate for 20 hours on sterilized

moist filter paper in Petri dishes in darkness at 20°C. At this time when the primary roots were 2-3 mm long uniform plants were selected and were transferred to sterilized agar slopes in test tubes containing auxin extract, one seed in each test tube (see fig.1). They were allowed to grow for a standard experimental period of 72 hours in the dark at 20°C. At the end of this period the length of the longest root of each plant was measured to the nearest mm. The rye seedling at this stage has 4 to 6 roots. The mean length of the longest root (primary root) was used as an index of root growth since it had been found to bear a constant relation to the total root growth (Lane 1936 and others), this is shown in table (4) p 28. The length of coleoptile and other roots also were recorded when possible.

Computation of the Result.

To calibrate the results, the inhibition of root length produced by a range of concentrations of indoleacetic acid was determined. In table (3) p 27 and fig.(2) the length of inhibited roots expressed as percentage of control roots, is related to the logarithm of the concentration of indoleacetic acid (mg per litre). Between 1.0 and 0.001 mg per litre of indoleacetic acid the relationship with inhibition is approximately linear. No consistent acceleration of root growth was noted at any concentration of indoleacetic acid which supports the observation of Åberg (1950, 1951, 1952), and also of Bonner and Koepfli (1939), Marmer (1937).

From the calibration curve thus obtained the IAA equivalent of a given plant extract could be determined.

An example will clarify the method used.

Suppose an extract was made of 10 embryos in 100 cc dilution.

The mean length of the longest root of 15 test plants in that extract was 48 mm and corresponding root length of control (growing on aqueous agar only) was 60 mm. Therefore, root length of test plant is 80 per cent of that of control plants. Inspection of the calibration curve shows 80% root growth was obtained with IAA at 0.0038 mg per litre (obtained from the anti logarithm of the direct reading). Therefore, extract of 10 embryos in 100 cc contain auxin, equivalent to 0.0038 mg/l IAA, that is, 0.0038 μgm/cc IAA. Therefore total auxin in 10 embryos = 100 x 0.0038 μgm.

" " " " 1 embryo = 0.038 μgm.

Error of the Method

It has been noted that at lower concentrations of IAA the agreement between replicates was better than at higher concentrations (table 3); for instance, at 0.001 mg/l the standard error of six replicate estimations was 0.57%, while that for 0.01 mg/l was 0.91%. In ~~the most part of~~ this work the dilutions of auxin extracts were so made that their corresponding equivalent amount of auxin were generally calibrated from the curve between 0.01 to 0.001 mg/l IAA where error was minimal.

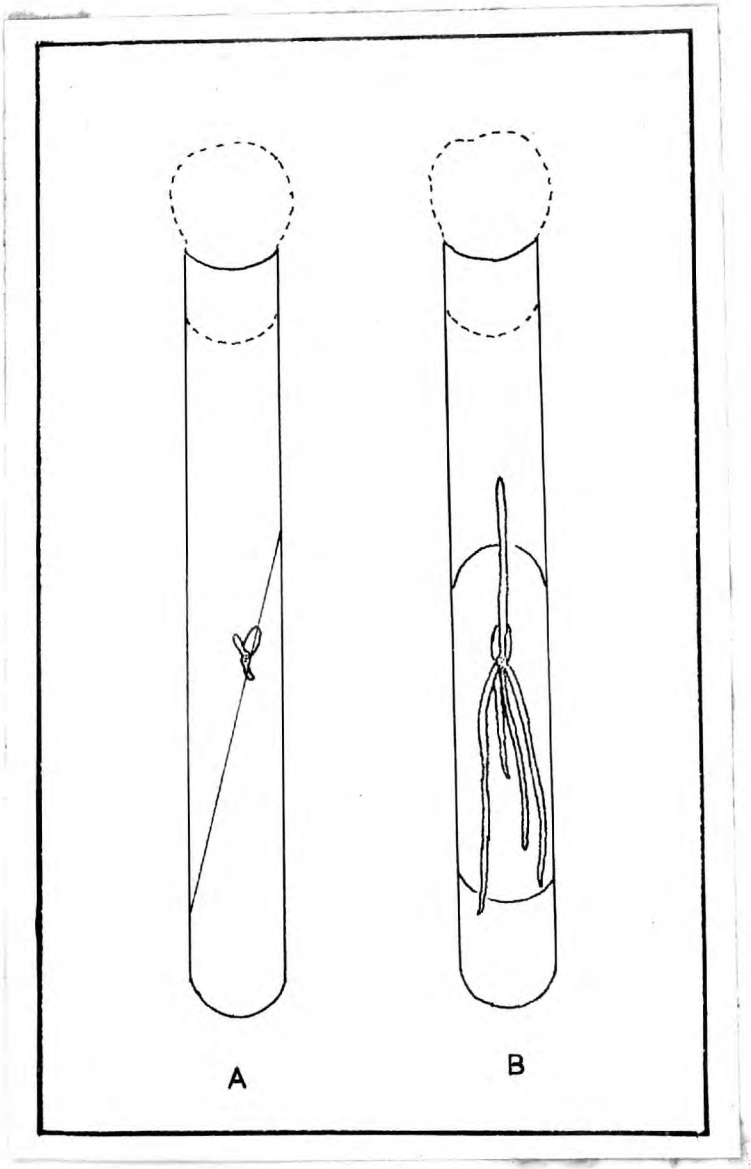

Figure 1. Culture of test plants. A. Position of germinating grain on agar slope containing auxin(side view). B.Root growth of test plant (front view).

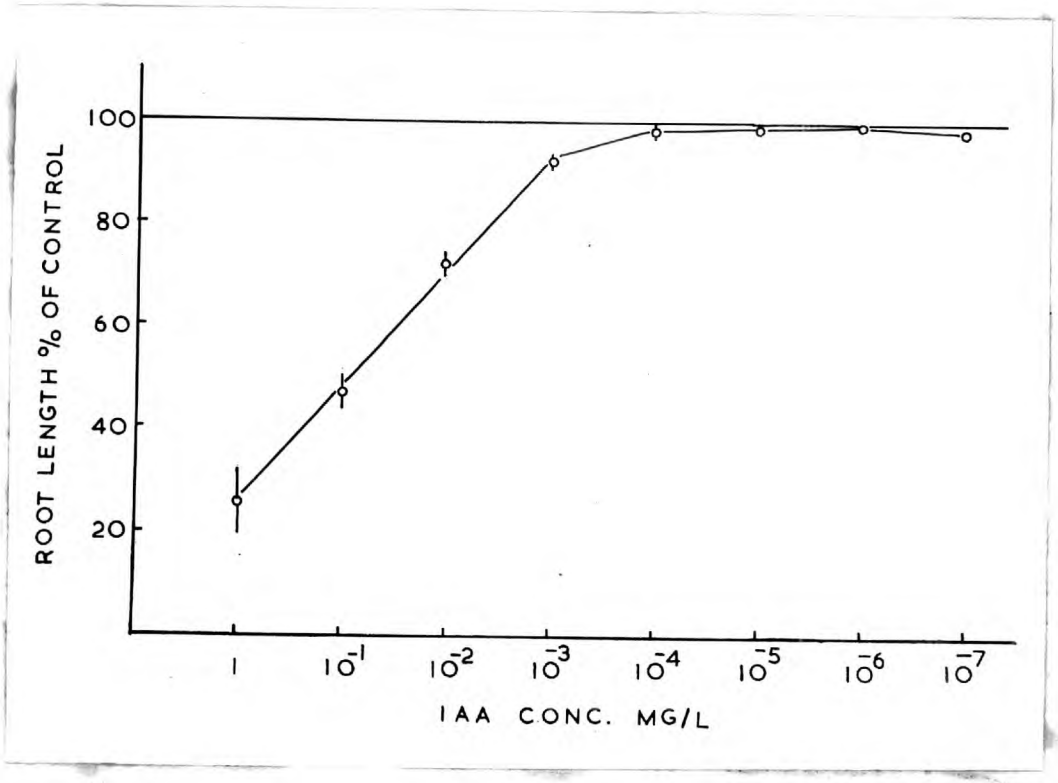

Figure 2. Calibration curve. Elongation (as percentage of control) of the longest root of the seedling of Petkus winter rye plotted against the log.concentration (mg/l) of indoleacetic acid. Each circle represents the mean of 6 determinations each on 10-15 test plants. The vertical lines through each mean extend to ± the standard deviation on each side.

Extraction of auxin.

Numerous workers including Boysen Jensen (1936), Went (1937), Van Overbeek (1938, 1945), Laibach and Meyer (1935), Thimann, Skoog and Bayer (1942), du Buy (1938), Haagen-Smit (1942), Avery et al (1939 b, 1940, 1941 b, 1945), Gordon (1946), Hatcher (1945), Sircar and Das (1949), have tried different methods for extraction of auxin from plants using different solvents and different types of plant tissues. In general it has been shown that the same method cannot be employed in every case as the nature of the tissue in relation to the auxin yield is specific. Water and ether have been proved to be successful for extracting free or diffusible auxin from green tissues of grasses (Van Overbeek 1938, 1945), woody stems of apple and plum (Hatcher 1948). In the case of the grass stem, heating of plant material prior or during the extraction was shown to be undesirable because it caused the release of substances antagonistic to auxin, therefore interfering with Avena test; furthermore heat is said to destroy an enzymic system responsible for the liberation or activation of auxin (Thimann and Skoog 1940, Van Overbeek 1945).

Some plant auxin is found in association with protein molecules and is readily released by alkali (NaOH at pH 9.5) (Gordon and Wildman 1943, Avery et al 1945, Gordon 1946) by proteolytic enzymes (Skoog and Thimann 1940, Haagen-Smit et al 1942, Wildman and Gordon 1942) and by acids (Avery et al 1945, Gordon 1946).

Laibach and Meyer (1935) extracted auxin with hot acidulated alcohol from Zea and Helianthus seedlings and pointed out that precautions should be taken against the destruction of auxin by oxidative enzymes of the plant during and after extraction. Such enzymatic inactivation of auxin also has been demonstrated by Tang and Bonner (1948) and others.

In the present study [illegible], auxin has been extracted either by water or by ether at low temperature (0 °C) over a long period. The details of the extraction process is as follows:- The plant material in question e.g., embryos, stems, apices and ears was dissected out, its length measured and weighed accurately to nearest mg. The material was then cut into pieces (approx. 2 mm cubes) and put into about 3 times its own volume of water or ether. After storage for 48 hours at a temperature of 0 °C the extract was filtered off and more water was added to material followed again by filtration. In the case of ether extract the ether was evaporated off first by placing the tubes in a bath of warm water and afterwards water was added. The filtrate was neutralized, if necessary with N/200 NaOH and diluted with phosphate buffer at ca pH 5.9, (1 Na_2HPO_4 + 9 KH_2PO_4 total concentration was 6.7 mM) which was also used for control seedlings and for IAA Solns. (In experiment 4 no buffer was used).

The filtrate was then diluted to either 50 cc or 500 cc of water according to the amount of plant material and an equal volume of 1.6% solution of agar added giving as the final dilution either 100 cc or 1000 cc. The 0.8% agar - extract was then poured into 5" x 5/8" test tubes each receiving 6.25 cc of liquid, the tubes were plugged and autoclaved at 120° C for 15 minutes and were slanted at an angle about 85°. The tubes for control seedlings containing aqueous agar or different concentrations of indoleacetic acid were also similarly sterilized by autoclaving at 120° C for 15 minutes.

For each determination duplicate extracts were made; for each extract the tissue in question was dissected from a sample of 10 plants (in case of plant apices 20 or 40) and its auxin content determined by its root inhibiting effect on 10 - 15 test plants.

Light Conditions of out door experiments.

Four different daylength were employed.

1. Continuous light.
2. Constant 17 hours long day.
3. Normal summer day (long day)(June to September 1952).
4. 8 hours day length (short day).

Continuous light was provided by supplementing natural day length with artificial light from six 60 watt tungsten filament lamps, giving 25 to 40 foot candles at pot level.

Constant 17 hours day length was provided by supplementing natural day length with 60 watt tungsten filament bulbs. The intensity at pot level was 20 - 30 foot candles with the 7 hours dark period centred at midnight.

Normal summer day length was 14½ hours at the time of planting out, rising to 16½ hours at the end of June, and falling to 13 hours by the end of the experiment.

Short day conditions (short days) were provided by covering each group of 9 pots with a duralimin cubical box with 3' sides. The open edges at ground level were made light-tight by covering with sand.

Temperature.

Temperature records were kept as far as possible. In dark room where excised embryos and test plants were cultured, 20°C ± 1 was maintained. The fluctuation of temperature was over a wide range (10° - 25°C) in outside where the outdoor experimental plants were grown. During summer days specially within a few days of planting, the temperature was carefully kept below 20°C by spraying the pots with water to avoid devernalisation at high temperature. During cooler nights at the end of summer the greenhouse temperature was maintained at approx. 15°C.

Vernalisation Technique.

The Russian technique with limited moisture was used for vernalising the grain. The method used in the present work is essentially the same as that described by Purvis and Gregory (1952) and Friend (Ph.D. Thesis L.U. 1953). The air-dry grain of Petkus winter rye was dusted with 'Spergon'(Tetrachloro para-benzoquinone) to prevent fungal contamination and soaked in sterilized distilled water for 6-7 hours; then they were placed in weighed vernalisation dishes (described by Purvis and Gregory 1952) and sprouted for 24 hours at 20°C. During the subsequent treatment the dishes were kept in the refrigerator at 1-2°C and were periodically weighed so that water content (50% of the oven dry weight of the grain) could be maintained by the addition of sterilized water to replace that lost by evaporation.

Culture of excised embryos.

Unless otherwise stated excised embryos were cultured in all experiments on sterilized nutrient agar slopes containing 2% sucrose and adequate mineral nutrients by the method developed and extensively used by Gregory and Purvis (1938,1944).

The composition of the medium was as follows:-

TABLE 1

Medium for Culture excised embryos.

Solution A			
$MgSO_4$			1.48 gm.
KCl			1.30 gm.
KH_2PO_4			0.24 gm.
Water			1000 cc.
Solution B			
$Ca(NO_3)_2$	anhydrous	...	1.9 gm.
KNO_3	...	...	1.62 gm.
water	...	...	1000 cc.
Solution C			
$FeSO_4$	...	...	0.24 gm.
water	...	...	100 cc.

For 100 cc medium:-

5 cc	A
5 cc	B
0.4 cc	C
2 gm	Sucrose
0.8 gm	Agar
90 cc	water.

Sand Culture Technique

The plants were grown in sand culture in unglazed earthenware 10 inch pots, the sand being watered with the nutrients used by Purvis and Gregory. The nutrient solution

was applied weekly to each pot until signs of shooting appeared, when feeding was discontinued. The composition of the nutrient is as follows:-

TABLE 2

Sand culture nutrient for each pot.

1. Major Element.	gm. per week (approx.).
Na_2HPO_4	0.32
$MgSO_4$	0.16
K_2SO_4	0.23
$NaNO_3$	1.14
2. Minor Elements.	**gm. per 2 weeks.**
$FeCl_3$	0.25
$MnSO_4$	0.002
H_3BO_4	0.003
$CuSo_4, 5H_2O$	0.0003
$ZnSO_4, 7H_2O$	0.0003

Calcium was omitted because of the high amount in London tapwater, with which the pots were watered daily.

TABLE 3

Relation of elongation of longest root (primary root) of the seedling of Petkus winter rye to the concentration of Indoleacetic acid.

I.A.A. mg/l	Mean length of longest root. mm.	Mean length of longest root in control mm.	Mean length of longest root as % of control.	Mean	Standard error ±	Standard deviation ±
1	20.3 ± 3.1	53.5 ± 2.2	37.94			
"	14.8 2.5	"	27.66			
"	13.5 1.6	55.6 1.7	24.28	26.3	2.6	6.4
"	13.7 1.7	"	24.64			
"	10.9 1.7	58.2 2.4	18.72			
"	14.3 2.7	"	24.57			
10-1	25.4 ± 1.6	53.5 ± 2.2	47.47			
"	28.9 1.9	"	54.00			
"	25.6 2.4	55.6 1.7	46.04	47.98	1.5	3.6
"	25.1 2.6	"	45.14			
"	29.4 1.6	58.2 2.4	50.51			
"	26.1 3.1		44.84			
10-2	37.6 ± 3.0	53.5 ± 2.2	70.28			
"	38.2 1.6	"	71.40			
"	38.9 2.8	55.6 1.7	69.96	72.1	0.91	2.2
"	40.5 1.6	"	72.84			
"	40.0 1.4	58.2 2.4	72.16			
"	44.3 2.5	"	76.11			
10-3	50.3 ± 3.3	53.5 ± 2.2	94.01			
"	48.7 1.5	"	91.02			
"	51.1 3.8	55.6 1.7	91.90	92.7	0.57	1.4
"	52.5 2.4	"	94.42			
"	54.3 1.0	58.2 2.4	93.29			
"	53.3 1.1	"	91.58			
10-4	53.1 ± 2.5	53.5 ± 2.2	99.25			
"	54.8 1.7	55.6 1.7	98.56			
"	56.7 1.8	58.2 2.4	97.42	97.9	0.68	1.4
"	56.0 2.7	"	96.21			
10-5	53.0 ± 3.1	53.5 ± 2.2	99.06	99.06		
10-6	53.2 1.4	53.5 2.2	99.43	99.43		
10-7	52.9 2.5	53.5 2.2	98.87	98.87		

TABLE 4

Relation of elongation of roots and coleoptile of the seedling of Petkus winter rye to the concentration of Indoleacetic acid.

Sample	IAA mg/l	Mean length mm I	Mean length mm II	Mean Length of control mm	Mean Length as % of control I	Mean Length as % of control II
Total of all Roots	1	46.9	43.9	178.7	26.2	24.6
1st Longest Root	"	20.3	14.8	53.5	37.9	27.7
2Hd " "	"	11.4	11.9	46.7	24.4	25.5
3rd " "	"	8.2	9.1	38.6	21.2	23.6
4th " "	"	5.6	7.0	29.8	18.8	23.5
Coleoptile	"	16.7	15.7	31.5	53.0	49.8
Total of all Roots	-1 10	79.9	99.0	178.7	44.7	55.4
1st Longest Root	"	25.4	28.9	53.5	47.5	54.0
2nd " "	"	20.2	27.0	46.7	43.3	57.8
3rd " "	"	15.6	22.5	38.6	40.4	58.3
4th " "	"	12.7	17.1	29.8	42.6	57.4
Coleoptile	"	23.2	25.6	31.5	73.7	81.3
Total of all Roots	-2 10	121.9	122.6	178.7	68.2	68.6
1st Longest Root	"	37.6	38.2	53.5	70.3	71.4
2nd " "	"	28.3	28.2	46.7	60.6	60.4
3rd " "	"	24.9	24.6	38.6	64.5	63.7
4th " "	"	21.3	20.6	29.8	71.5	69.1
Coleoptile	"	29.4	25.3	31.5	93.3	80.3
Total of all Roots	-3 10	156.5	165.1	178.7	87.6	92.4
1st Longest Root	"	50.3	48.7	53.5	94.0	91.0
2nd " "	"	44.3	46.3	46.7	92.7	99.1
3rd " "	"	37.3	35.6	38.6	96.6	92.2
4th " "	"	29.0	29.1	29.8	97.3	97.7
Coleoptile	"	28.3	32.2	31.5	89.8	102.2

I , II Replicate one and two each determined by 10 Plants.

1. Auxin content of excised embryos of different ages in relation to the concentration of sucrose in the medium

The concentration of sugar is one of the critical factors in the culture of excised embryos in vitro (Purvis 1944, 1947). Without sugar the growth and vernalisation response of excised embryos is limited and increases with the concentration of sugar up to 2 percent. High concentration of sugar also diminish the growth rate, but the vernalisation response does not seem to change with the decreased growth rate (Friend. 1953. Ph.D. thesis L.U). These investigations show that the part played by sugar in vernalisation and in growth are quite independent. This dependence of growth on the concentration of sugar indicates the need for a study of the auxin content of excised embryos in relation to the concentration of sugar in the medium.

Culture media were prepared (Table 1) with concentration of sucrose ranging from 0 to 10 percent. Embryos of Petkus winter rye were excised (Gregory and Purvis 1938) and cultured for 2, 4 or 8 days at 20 °C in the dark.

Auxin was extracted by water as described on page 21 to 22 of this paper. 10 embryos were used for each 100 cc extract. In table (7) the data for duplicate series of excised embryos grown on various concentrations of sucrose are given. The growth data include shoot and root length and fresh weight of the embryos; the auxin data are presented in terms of total auxin as equivalent IAA in μgm per embryo, and the concentration of auxin as μgm per gm., of fresh weight. The data are given in graphical form in figures [illegible] (4B - C).

The effect of sucrose concentrations on the growth of the coleoptile, leaf and root are given in figure (3A - B). The measurements were made daily during growth using inactive red light. It is evident that growth increases with the sucrose concentration up to two percent sucrose. In ten percent sucrose however the growth of the excised embryo decreases. The form of all the curves for embryo growth in all series is similar. Maximal growth rate of coleoptile occurs at about 3 days from germination and in 5-6 days it reaches its final length. At this point the first foliage leaf continuing its growth, breaks through the tip of the coleoptile. It will be noted that the growth of the foliage leaves is also increased by rising the sucrose concentration.

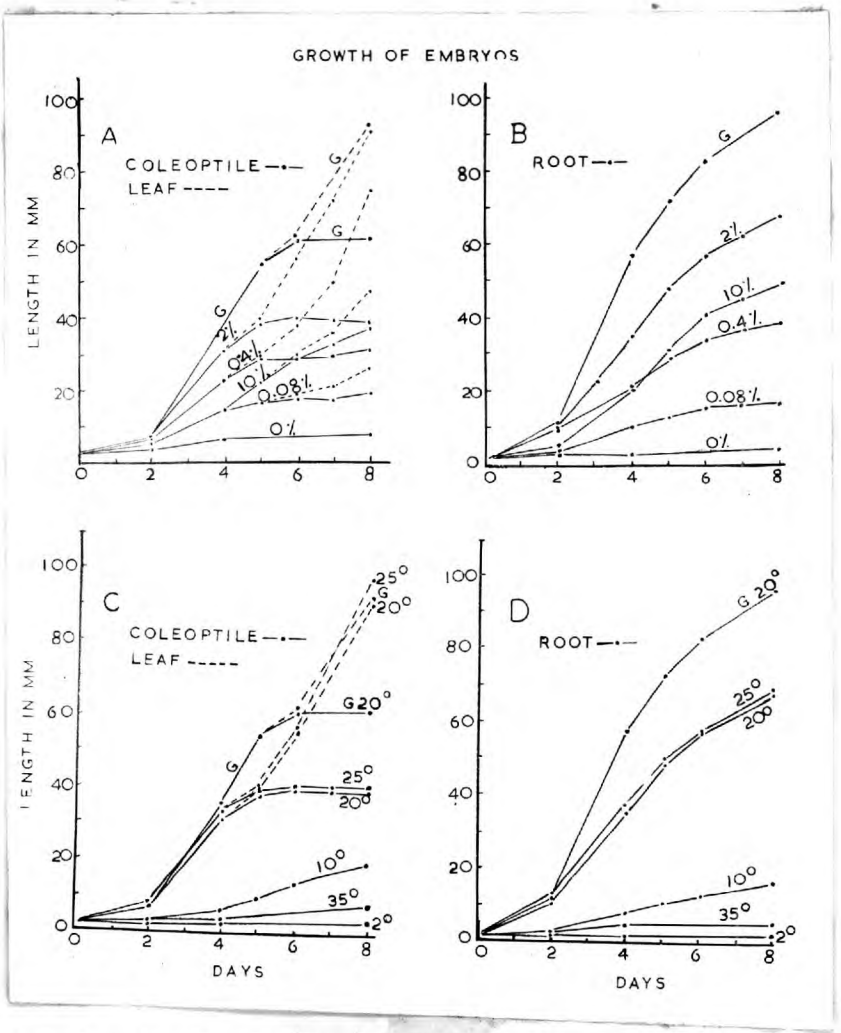

Figure 3. A. B. Effect of sucrose concentration on growth of coleoptile, leaf and root of excised embryos in the dark at 25°c. C. D. Effect of temperature (°C) on growth of coleoptile, leaf and root of excised embryos in the dark on 2% sucrose.(G, embryo growing attached to the endosperm on aqueous agar.)

TABLE 5

Effect of preliminary soaking on auxin content of excised embryos of Petkus winter rye immediately after excision. Auxin was extracted from duplicate samples each of 10 excised embryos in 100 cc of water.

10 Excised Embryos	Embryo length mm	Fresh wt. gm	Root length test plant in % of control	Total Auxin IAA Eq. µgm per embryo	Auxin conc IAA Eq. µgm per gm. wt.
Embryos from grain soaked for 2 hours	2.7	0.025	94.0	0.007	2.50
	2.8	0.020	97.7	0.002	1.00
Embryos from grain soaked for 5½ hours	3.0	0.025	95.5	0.004	1.60
	3.0	0.030	93.7	0.007	2.33

TABLE 6

Growth and auxin content of embryos of Petkus winter rye grown at 20°C in dark without excision. Auxin was extracted from duplicate samples each of 10 excised embryos in 100 cc of water.

Growth of Embryos				Auxin content		
Days old	Shoot length mm	Root length mm	Fresh wt. gm.	Root length test plant in % of control	Total Auxin IAA Eq. µgm per embryo	Auxin conc. IAA Eq. µgm pe gm.wt.
2	5.4	9.8	0.10	86.0	0.020	2.00
"	5.8	10.3	0.08	88.2	0.016	2.00
4	30.9	52.4	0.48	76.1	0.058	1.20
"	28.0	50.6	0.64	78.7	0.044	0.69
8	88.4	98.6	1.02	73.0	0.079	0.77
"	88.2	97.1	1.30	72.0	0.088	0.68

With regard to auxin production by excised embryos, the following facts are apparent. In the absence of endosperm the embryo on a sucrose medium can produce auxin in a concentration equal that found in embryos still attached to the endosperm (c.f. Tables 6, 7). The total amount of auxin produced by excised embryos is however less than that of unexcised embryos. Sugar was found necessary for the production of auxin in the excised embryo; in absence of sucrose the auxin content did not increase in 8 days. The mean rate of auxin accumulation in the embryo progressively increased with the concentration of sucrose and was found to bear a approximately linear relationship with the mean growth rate up to 4 days (Figure 40), after 4 days auxin production ceased to keep pace with growth. This was especially notable in embryos on suboptimal sucrose concentrations.

In all series except that without sugar auxin accumulated most rapidly between the second and fourth day. This accumulation of auxin in excised embryos was greatest on 2% sucrose. Lower concentrations (0.08% and nil), greatly reduced the production of auxin. 10% sucrose concentration also did not increase the production of auxin over that of 2%, hence 2% sucrose concentration which was optimal for growth also proved to be optimal for production of auxin.

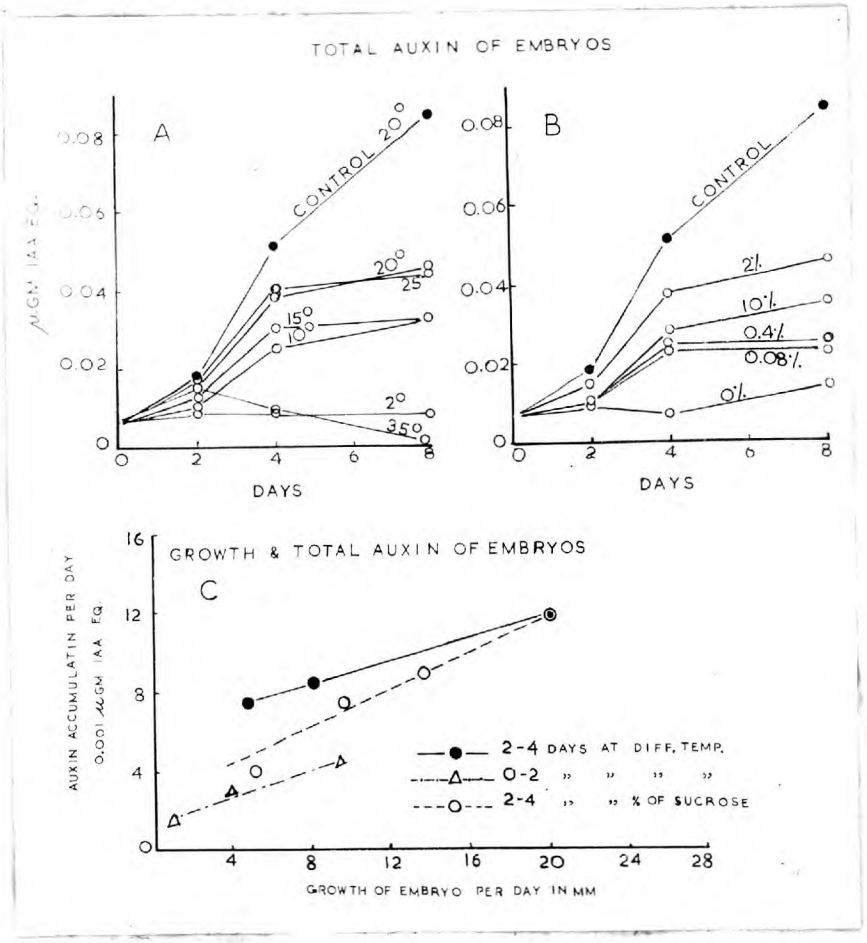

Figure 4. A. Effect of temperature (°C) on the total auxin content of excised embryos.(Control : the embryo growing attached to the endosperm in aqueous agar at 20°C). B. The effect of sucrose concentration on the total auxin content of excised embryos.(Control : the embryo growing attached to the endosperm in aqueous agar at 20°C). C. Relation between growth of excised embryos and auxin production at different temperatures or in different concentrations of sucrose for 0 to 4 days.

TABLE 7

Effect of concentration of sugar in the medium on growth and auxin content of excised embryos of Petkus winter rye. Auxin was extracted from duplicate samples each of 10 excised embryos in 100 cc of water.

Growth of Embryos						Auxin Content	
Sugar %	Days old	Shoot length mm	Root length mm	Fresh wt. gm	Root length test plant in p.c of control	Total Auxin IAA Eq. µgm per embryo	Auxin conc. IAA Eq. µgm per gm.wt.
	2	2.8	3.8	0.08	93.2	0.009	1.12
	"	4.7	7.0	0.10	92.5	0.011	1.10
10	4	10.5	17.5	0.25	80.3	0.037	1.48
	"	14.4	22.5	0.26	86.3	0.020	1.76
	8	66.0	45.5	0.68	80.7	0.036	0.52
	"	43.2	44.7	0.61	81.3	0.034	0.55
	2	6.0	10.2	0.13	90.1	0.014	1.07
	"	6.8	9.7	0.13	89.0	0.015	1.15
	4	29.3	30.3	0.32	79.2	0.042	1.31
2	"	27.7	26.7	0.36	80.9	0.035	0.97
	8	98.2	43.3	0.62	78.0	0.047	0.75
	"	96.9	58.9	0.68	79.0	0.043	0.63
	2	6.5	11.3	0.13	92.7	0.010	0.76
	"	6.2	8.3	0.09	92.7	0.010	1.11
0.4	4	25.9	17.9	0.29	83.2	0.028	0.96
	"	20.4	10.8	0.18	85.7	0.021	1.16
	8	64.4	30.4	0.35	87.2	0.018	0.51
	"	61.9	35.4	0.38	81.9	0.032	0.84
	2	5.1	3.2	0.08	92.8	0.010	1.25
	4	17.1	8.5	0.14	84.7	0.023	1.64
	"	9.1	2.4	0.10	85.0	0.023	2.30
0.08	8	25.9	9.4	0.21	88.5	0.016	0.76
	"	24.7	15.6	0.20	82.1	0.031	1.55
	"	24.3	11.9	0.22	85.0	0.023	1.04
	2	3.1	2.3	0.07	93.7	0.007	1.00
		3.2	1.9	0.08	92.9	0.010	1.25
0	4	6.4	1.5	0.07	94.0	0.007	1.00
		4.9	2.3	0.07	93.8	0.007	1.00
	8	7.4	4.1	0.11	90.0	0.014	1.27
		5.9	3.	0.10	90.8	0.013	1.30

Since the amount of auxin increase paralleled the growth of the embryos during the first four days, there was little change in its concentration, but between 4 to 8 days the concentration of auxin in all series was approximately halved. It is only in embryos with attached endosperm that total auxin increased after 4 days (Table C), apart from a possible small increase in those on 2% sucrose.

In suboptimal sugar concentrations, the lack of increase of auxin from 4 to 8 days accompanied by ~~steady~~ growth rate of the whole embryo (Fig 3c) requires consideration. Accordingly, a detail study of the relation of auxin to the growth of different parts of the embryo (e.g. coleoptile, coleoptile node, leaf, root) grown on two different concentrations of sucrose for 3, 4 , 6 or 8 days was carried out. The results are expressed in tables (8 to 11) and figures (5 to 8), which show that in early stages of growth (3-day old embryos) irrespective of treatments most of the auxin of the embryo was in the coleoptile and coleoptile node (Figure 8), and increases in quantity in coleoptile during the next 24 hours with the growth of coleoptile (Figure 6). At the age of 5-6 days the tip of the coleoptile was broken followed by complete termination of coleoptile growth and auxin accumulation in it. 8 day old coleoptiles in some cases showed less total auxin than was found at four days.

TABLE 8

Effect of concentration of sugar in the medium on growth and auxin content of coleoptiles of Petkus winter rye. Auxin was extracted from duplicate samples each of 10 coleoptiles in 100 cc water.

Growth of Coleoptiles					Auxin Content	
Treatment	Days old	Length mm.	Fresh wt. gm.	R.T.P. %	Total Auxin IAA Eq. µgm per coleoptile	Auxin Conc. IAA Eq. µgm per gm. wt.
	3	14.3	0.100	92.1	0.011	1.10
	"	12.6	0.090	91.2	0.012	1.33
Embryo without excision	4	36.0	0.260	85.0	0.023	0.88
	"	33.4	0.290	86.9	0.019	0.66
	6	63.7	0.400	87.1	0.019	0.48
	"	66.6	0.410	83.7	0.026	0.63
	8	66.5	0.395	87.9	0.017	0.43
	"	66.1	0.390	89.6	0.015	0.38
	3	10.1	0.090	92.3	0.011	1.22
	"	9.3	0.050	93.0	0.010	2.00
Excised embryo in 2% Sugar	4	25.0	0.120	88.5	0.016	1.33
	"	25.1	0.120	89.8	0.014	1.16
	6	43.9	0.190	92.6	0.010	0.52
	"	48.3	0.200	87.2	0.018	0.90
	8	45.6	0.220	90.0	0.014	0.63
	"	44.0	0.120	---	---	---
	3	8.9	0.040	90.6	0.013	3.25
	"	8.6	0.035	97.9	0.002	0.57
Excised embryo in 0.4% Sugar	4	23.7	0.110	88.8	0.015	1.36
	"	23.2	0.100	91.7	0.012	1.20
	6	34.4	0.140	95.8	0.003	0.21
	"	36.5	0.140	93.7	0.008	0.51
	8	36.2	0.140	93.5	0.009	0.64
	"	35.7	0.130	98.1	0.001	0.08

R.T.P. Root length of test plants in percentage of control.

TABLE 9

Effect of concentration of sugar in the medium on growth and auxin content of coleoptile nodes of Petkus winter rye. Auxin was extracted from duplicate samples each of 10 coleoptile nodes in 100 cc of water.

Treatment	Growth of Coleoptile Nodes: Days old	Length mm.	Fresh wt. gm.	R.T.P. %	Auxin content: Total Auxin IAA Eq. µgm per nodes	Auxin conc. IAA Eq. µgm. gm. wt.
Embryo without excision	3	2.0	0.015	95.3	0.004	2.67
	"	2.1	0.015	103.7	0	0
	4	2.3	0.035	92.3	0.011	3.14
	"	2.2	0.030	88.2	0.016	5.33
	6	2.3	0.030	96.1	0.003	1.00
	"	2.8	0.045	94.4	0.006	1.33
	8	2.8	0.040	91.6	0.012	3.00
	"	2.5	0.035	93.8	0.007	2.00
Excised embryos in 2% Sugar	3	2.4	0.025	94.9	0.005	2.00
	"	2.3	0.025	93.2	0.009	3.60
	4	2.4	0.025	96.4	0.003	1.20
	"	2.7	0.040	93.4	0.009	2.25
	6	2.6	0.030	92.6	0.010	3.33
	"	2.6	0.030	92.6	0.010	3.33
	8	2.7	0.030	91.2	0.012	4.00
	"	2.7	0.035	97.7	0.002	0.57
Excised embryo in 0.4% Sugar	3	2.2	0.010	94.2	0.006	6.00
	"	2.2	0.010	93.8	0.007	7.00
	4	2.3	0.020	94.6	0.005	2.50
	"	2.5	0.025	95.6	0.004	1.60
	6	2.5	0.025	93.1	0.010	4.00
	"	2.6	0.030	95.8	0.003	1.00
	8	2.4	0.025	92.9	0.010	4.00
	"	2.4	0.040	90.2	0.013	3.25

R.T.P. Root length of test plants in percentage of control.

TABLE 10

Effect of concentration of sugar in the medium on growth and auxin content of leaves of Petkus winter rye. Auxin was extracted from duplicate samples each of 10 leaves in 100 cc of water.

Treatment	Growth of leaves: Days old	length mm	Fresh wt. gm.	R.T.P. %	Auxin content: Total Auxin IAA Eq. µgm. per Leaves	Auxin conc. IAA Eq. µgm. per gm. wt.
	3	10.8	0.030	99.1	0	0
	"	10.3	0.025	99.4	0	0
Embryo with-	4	29.8	0.105	91.4	0.012	1.14
out excision	"	28.9	0.110	96.2	0.003	0.27
	6	65.3	0.290	81.1	0.034	1.17
	"	65.3	0.300	85.5	0.022	0.73
	8	89.2	0.485	76.3	0.056	1.15
	"	85.9	0.395	83.1	0.028	0.71
	3	4.6	0.010	98.0	0	0
	"	3.5	0.010	99.3	0	0
Excised	4	13.2	0.025	99.2	0	0
embryo in	"	13.6	0.030	99.5	0	0
2% Sugar						
	6	53.4	0.175	92.4	0.011	0.63
	"	53.7	0.160	93.0	0.010	0.63
	8	76.2	0.290	83.7	0.027	0.93
	"	75.8	0.270	87.8	0.017	0.63
	3	3.6	0.010	101.9	0	0
	"	3.1	0.008	99.6	0	0
Excised	4	11.7	0.030	96.2	0.003	1.00
embryo in	"	12.3	0.025	---	---	---
0.4% Sugar						
	6	44.3	0.100	93.5	0.009	0.90
	"	41.0	0.100	92.5	0.010	1.00
	8	58.3	0.120	88.5	0.016	1.33
	"	48.7	0.110	92.9	0.010	0.91

R.T.P. Root length of test plant in percentage of control.

TABLE 11

Effect of concentration of sugar in the medium on growth and auxin content of roots of Petkus winter rye. Auxin was extracted from roots of duplicate samples each of 10 embryos in 100 cc of water.

Growth of Roots					Auxin Content	
Treatment	Days old	Length mm	Fresh wt. gm.	R.T.P. %	Total Auxin IAA Eq. µgm. per embryo	Auxin conc. IAA Eq. µgm. per gm. wt.
	3	27.2	0.200	97.9	0.002	0.10
	"	26.8	0.135	99.1	0	0
Embryo without excision	4	58.2	0.31	84.6	0.023	0.74
	"	57.0	0.35	92.7	0.010	0.29
	6	81.4	0.41	87.2	0.018	0.45
	"	83.8	0.45	86.3	0.020	0.49
	8	93.1	0.43	83.7	0.026	0.60
	"	94.0	0.48	82.4	0.030	0.63
	3	16.3	0.050	94.9	0.005	1.00
	"	11.1	0.045	97.4	0.002	0.44
Excised embryo in 2% Sugar	4	28.5	0.100	93.6	0.008	0.80
	"	29.5	0.105	96.3	0.003	0.29
	6	51.9	0.140	93.9	0.007	0.50
	"	54.1	0.140	91.3	0.012	0.86
	8	62.4	0.200	87.9	0.017	0.85
	"	60.1	0.205	86.5	0.019	0.93
	3	8.5	0.010	100.7	0	0
	"	9.1	0.015	102.9	0	0
Excised embryo in 0.4% Sugar	4	24.0	0.050	94.6	0.005	1.00
	"	23.9	0.040	98.3	0.002	0.50
	6	32.4	0.060	95.4	0.004	0.67
	"	31.2	0.055	94.2	0.006	1.09
	8	31.3	0.050	95.8	0.003	0.60
	"	30.4	0.045	98.1	0.001	0.22

R.T.P. Root length of test plant in percentage of control.

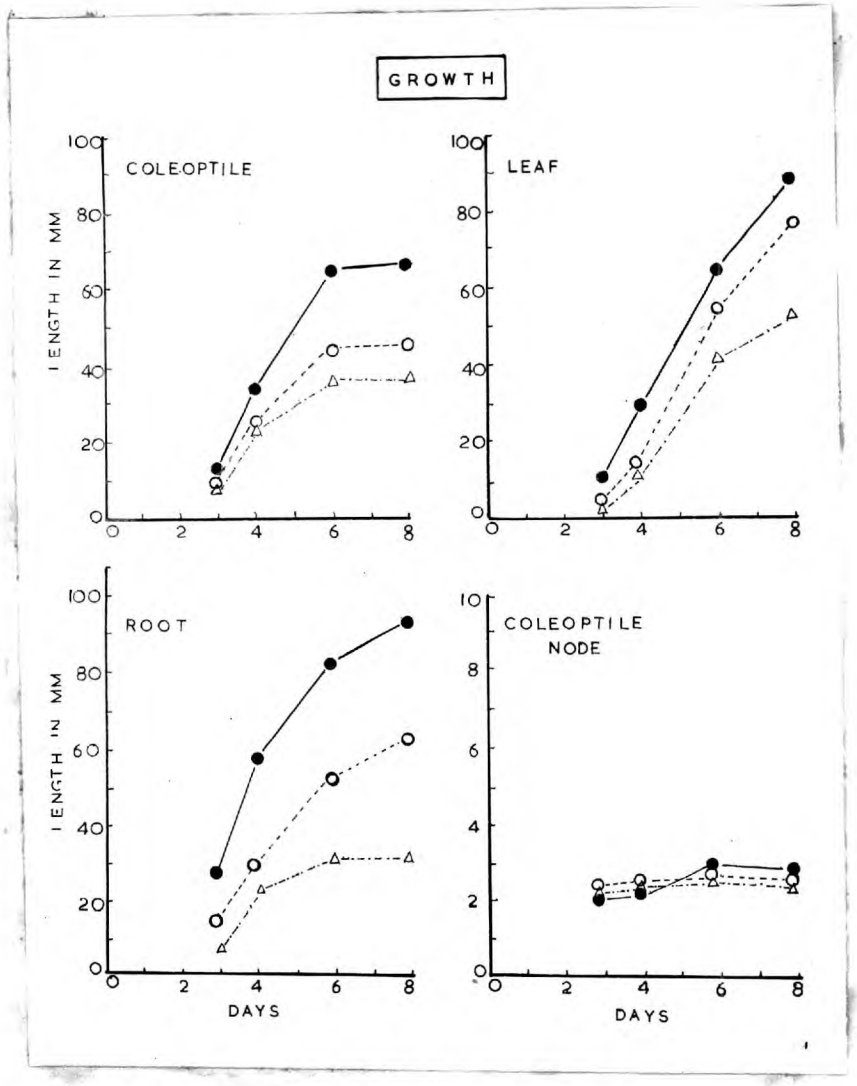

Figure 5. Growth in length of the coleoptile, leaf, root and the coleoptile node in the dark at 20°C. Closed circles, the embryo growing attached to the endosperm. Open circles, excised embryos in 2% sucrose. Triangles, excised embryos in 0.4% sucrose.

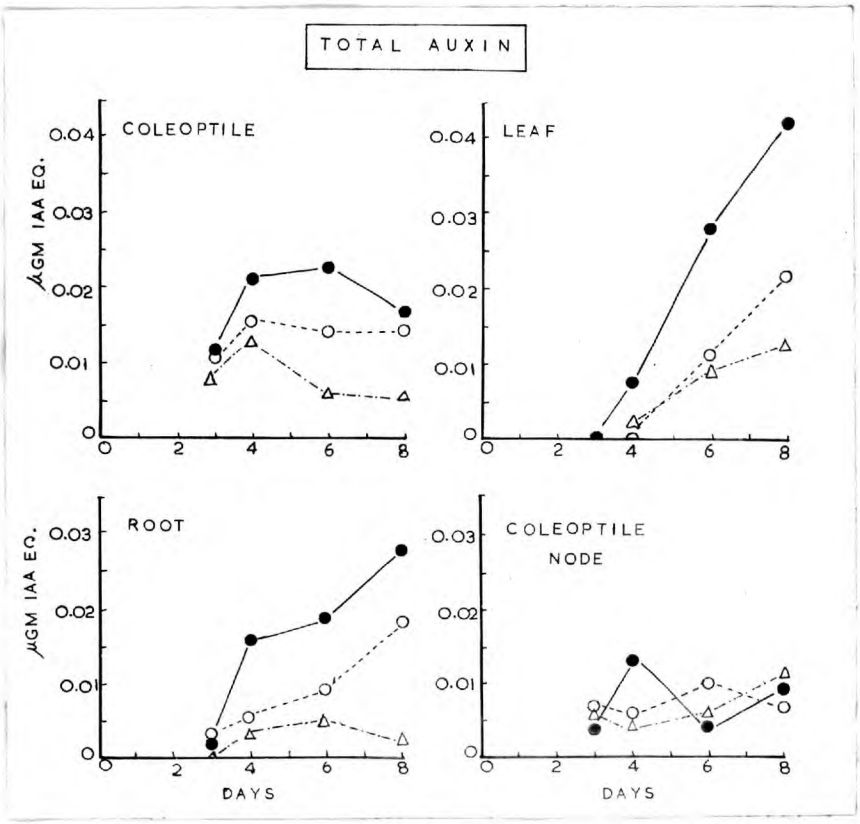

Figure 6. The total auxin content of the coleoptile, leaf, root and the coleoptile node in the dark at 20°C. Closed circles, the embryo attached to the endosperm. Open circles, the excised embryo in 2% sucrose. Triangles, the excised embryo in 0.4% sucrose.

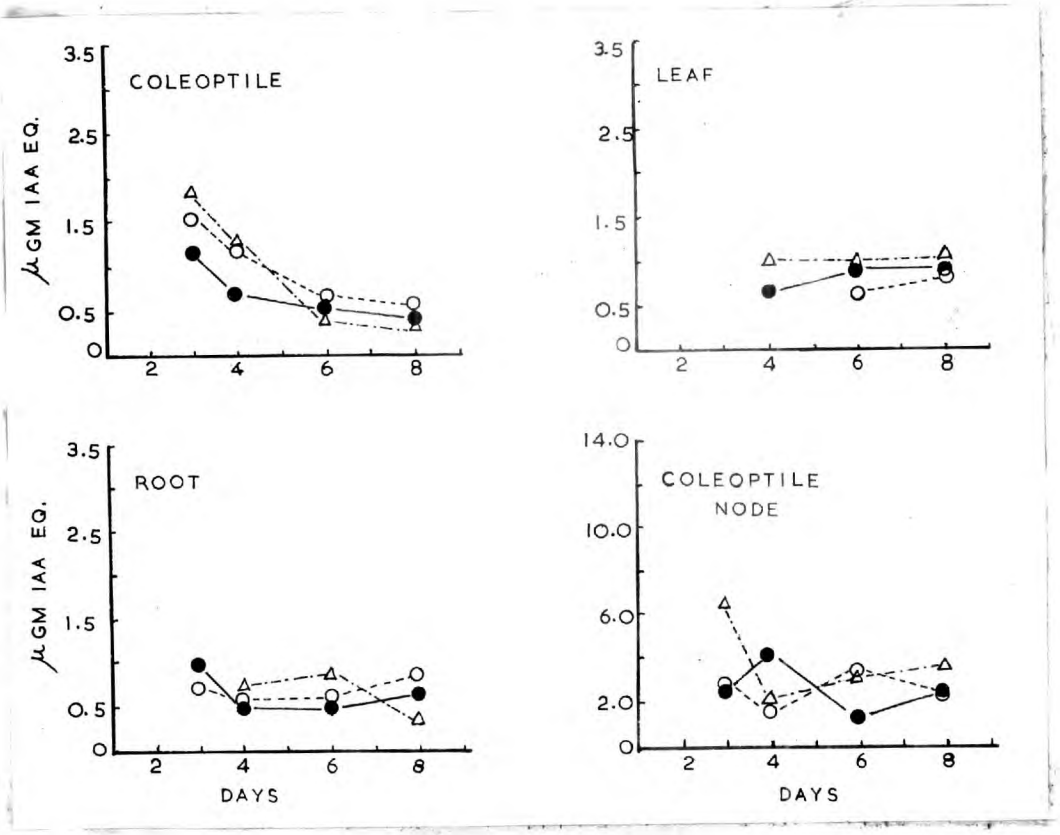

Figure 7. The concentration of auxin (auxin per gm. fresh weight) in the coleoptile, leaf, root and coleoptile node in the dark at 20°C.

Closed circles, the embryo growing attached to the endosperm. Open circles, the excised embryos in 2% sucrose. Triangles, excised embryos in 0.4% sucrose.

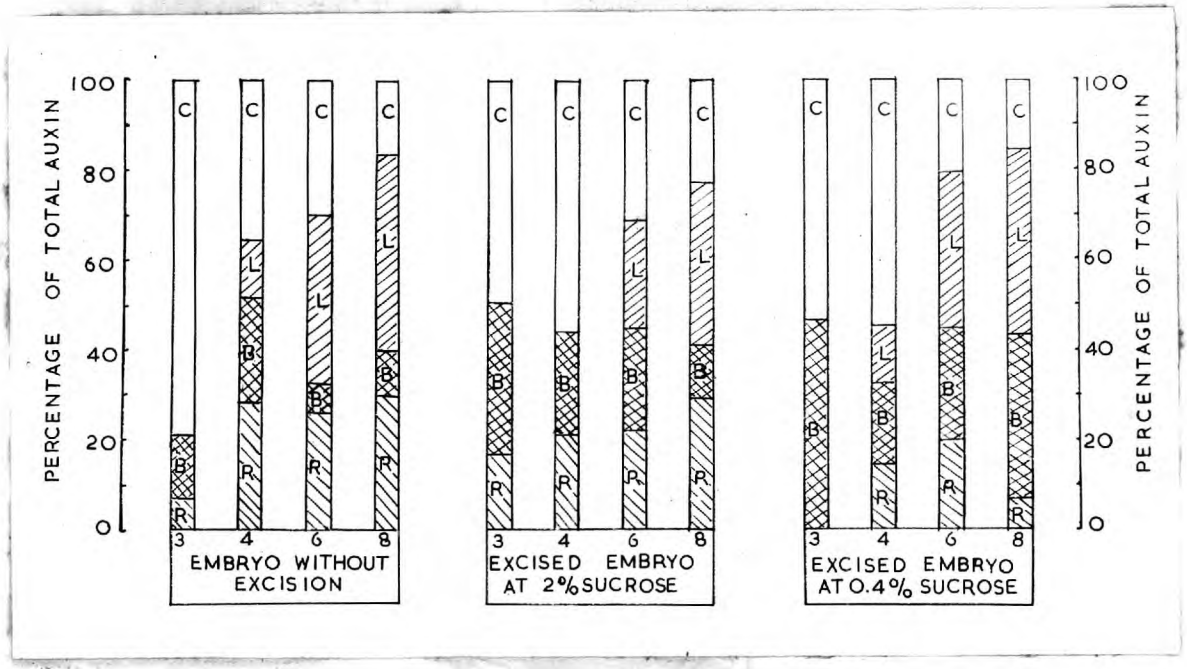

Figure 8. Percentage distribution of total auxin in the coleoptile (c), leaf (L), root (R) and coleoptile node (B) of (I) the embryo without excision, (II) excised embryos in 2% sucrose, (III) excised embryos in 0.4% sucrose after 3,4,6 and 8 days growth in the dark at 20°C.

on the other hand, the concentration of auxin in the coleoptile decreased with growth in all the treatments (Figure 7). The highest concentration of auxin among different parts of the embryo was found in the coleoptile node and remained almost unchanged during the period of eight days culture.

Auxin in leaves and roots increased rapidly with their growth, with the result that the percentage distribution of total auxin in relation to the whole of the embryo increased in leaf and root with growth (Figure 8). After termination of coleoptile growth most of the auxin of the embryo (about 70%) was found to be distributed in growing leaves and roots. The growth and total amount of auxin in the coleoptile, leaf and root of excised embryos on 2% sucrose were higher than that of embryos on 0.4% sucrose (Figure 5, 6) but less than that of embryos growing attached to the endosperm (control). It has also been noted that irrespective of treatments the concentration of auxin in the embryo did not differ greatly.

The study of the auxin content of the excised

embryo on 0.4% sucrose revealed that after 4 days from germination no auxin was accumulated either in coleoptile or root, (Fig 6) so that despite a small auxin increase in the leaf, the auxin level of the whole embryo remained practically unchanged between 4th and 8th day.

2. Auxin content of excised embryos of different ages in relation to the temperature.

Gregory and Purvis (1938) have shown that the excised embryos of rye can be cultured without the aid of the endosperm, in an agar medium containing sugar and can be vernalised by exposure to low temperature. These investigations indicate that although a considerable amount of auxin is present in the endosperm as shown by Hatcher (1945) in rye, Sircar and Das (1951) in rice, Avery et al (1926) [42] in maize and wheat, the vernalisation process of the plant is independent of the auxin present in the endosperm. The locus of the vernalisation of low temperature and devernalisation at high temperature (Friend, 1953. Ph.D. thesis. L.U.) seems to be in the tissues of the embryo itself.

These facts necessitate the study of the changes in auxin content of the excised embryos growing at different temperatures, including the vernalising, neutral and devernalising temperature.

In this experiment embryos of winter rye were excised under sterile conditions and cultured on a sterile agar medium containing 2% sucrose for periods of 2, 4 and 8 days at the following temperatures, 2°, 10°, 15°, 20°, 25°, and 35° centigrade. These temperatures were maintained in a refrigerator and a series of incubators. At the end of the determined period the auxin was extracted from duplicate samples, each of 10 embryos in 100 cc of water and determined by the method described on page 15-17 of this paper. Up to 25°C, growth in length of the coleoptile, leaf and root of the embryo and also the fresh weight increased with the temperature; above 25°C, the growth rate fell rapidly (Figure 3C - D). p31 The relation of temperature to the auxin content of the excised embryo is given in table (12) and figure (4a). Immediately after dissection from the grain the embryo contained about 0.005 μgm of auxin. (Table 5 p32 The rate of increase of this auxin in the embryo varied with the temperature. At 2°C (a temperature at which vernalisation occurs), auxin did not accumulate in the embryos throughout the 8 days of the experiment.

TABLE 12

Effect of temperature on growth and auxin content of excised embryos of Petkus winter rye grown on nutrient agar for different periods. Auxin was extracted from duplicate samples each of 10 excised embryos in 100 cc of water.

Growth of Embryos					Auxin Content		
T°C	Days old	Shoot length mm	Root length mm	Fresh wt. gm.	Root length test plant in % of control	Total Auxin IAA Eq. µgm per embryo	Auxin conc. IAA Eq. µgm per gm.wt.
	2	2.3	0.2	0.03	94.5	0.006	2.00
	"	2.4	0.4	0.04	92.4	0.010	2.50
2	4	2.6	0.5	0.04	95.3	0.005	1.25
	"	2.7	0.9	0.05	92.8	0.010	2.00
	8	2.9	1.6	0.05	94.9	0.006	1.20
	"	3.3	2.1	0.05	93.3	0.010	2.00
	2	2.7	1.9	0.06	9 5.1	0.005	1.00
	"	2.8	1.6	0.08	9 1.0	0.012	1.53
10	4	5.7	8.4	0.12	83.0	0.028	2.35
	"	5.5	8.1	0.10	85.4	0.022	2.24
	8	24.9	20.3	0.29	81.6	0.033	1.14
	"	18.0	16.0	0.21	82.5	0.030	1.40
	2	4.2	7.1	0.09	89.7	0.014	1.55
	"	4.2	5.7	0.08	91.1	0.012	1.50
15	4	14.8	18.6	0.20	82.2	0.030	1.58
	8	32.9	22.5	0.38	82.1	0.031	0.81
	"	40.5	49.9	0.60	81.8	0.032	0.53
	2	6.0	10.2	0.13	90.1	0.014	1.07
	"	6.8	9.7	0.13	89.0	0.015	1.15
20	4	29.3	30.3	0.32	79.2	0.042	1.31
	"	27.7	26.7	0.36	80.9	0.035	0.97
	8	88.2	43.3	0.62	78.0	0.047	0.75
	"	96.9	58.9	0.68	79.0	0.043	0.63
	2	7.4	10.5	0.19	87.0	0.019	1.00
	"	9.9	15.7	0.16	90.0	0.014	0.87
25	4	29.6	37.6	0.46	78.4	0.046	1.00
	"	35.9	37.4	0.41	81.4	0.034	0.82
	8	92.2	57.5	0.72	77.5	0.050	0.69
	"	106.1	55.8	0.93	80.6	0.036	0.38
	2	3.3	5.3	0.10	91.8	0.012	1.20
	"	2.0	0.8	0.05	87.9	0.017	3.40
35	4	3.8	6.2	0.11	91.5	0.012	1.09
	"	3.2	2.6	0.10	94.9	0.005	0.50
	8	10.7	6.8	0.16	98.1	0	0
	"	5.9	3.4	0.17	103.4	0	0
	"	-	-	0.10	101.1	0	0

At 10° C auxin accumulated throughout the period, increasing rapidly between the second and fourth days, a further small increase followed between the 4th and 8the day. The rate of increase was still greater at 15° C, 20° C and 25° C. At 35° C auxin accumulation ceased after 2 days and fell rapidly to zero. In the first four days the rate of accumulation of auxin at different temperatures was found to bear a linear relationship with growth rate (Figure 4C); after four days this relationship did not exist for growth continues while the rate of accumulation of auxin greatly decreased. These results agree with those of the first experiment with various sucrose concentrations.

It is of interest that at high temperature the auxin disappeared from the embryos; no extractable auxin was found in the embryos at the end of eight days culture at 35° C (Table 12). Accordingly a number of transfer experiments were carried out, which present a conclusive proof of the disappearance of auxin at 35° C. From table (13) and figure (9) it can be seen that 4-day old embryos cultured at 20° C when transferred to 35° C gradually lose their auxin content; in the period of 8 days culture at 35° C about 80 percent of the auxin found at 4 days ~~was~~ had disappeared (table 13). The remainder (0.007 μgm) was about equal to the initial amount found in the freshly dissected embryo. A small amount of growth also was noted in the first 4 days at 35° C after transfer from 20° C; but no growth was apparent in the ensuing 4 days. Other embryos

TABLE 13

Growth and auxin content of excised embryos transferred (A) from 20°C to 35°C or (B) from 35°C to 20°C after different periods. Auxin was extracted from duplicate samples each of 10 excised embryos in 100 cc of water.

Treatment	Embryo length mm	Fresh wt. gm	Root length test plant in % of control	Total Auxin IAA Eq. µgm per embryo	Auxin conc IAA Eq. µgm per gm.wt.
20°C for 2 days 35°C for 2 days	41.2	0.29	95.9	0.003	0.10
"	28.5	0.24	96.4	0.003	0.13
20°C for 4 days 35°C for 4 days	88.2	0.47	87.1	0.019	0.40
"	87.2	0.49	86.9	0.019	0.39
20°C for 4 days 35°C for 8 days	80.5	0.29	94.9	0.005	0.17
"	80.3	0.35	93.3	0.009	0.26
35°C for 4 days 20°C for 4 days	77.9	0.45	84.9	0.023	0.51
"	70.7	0.41	90.4	0.013	0.32

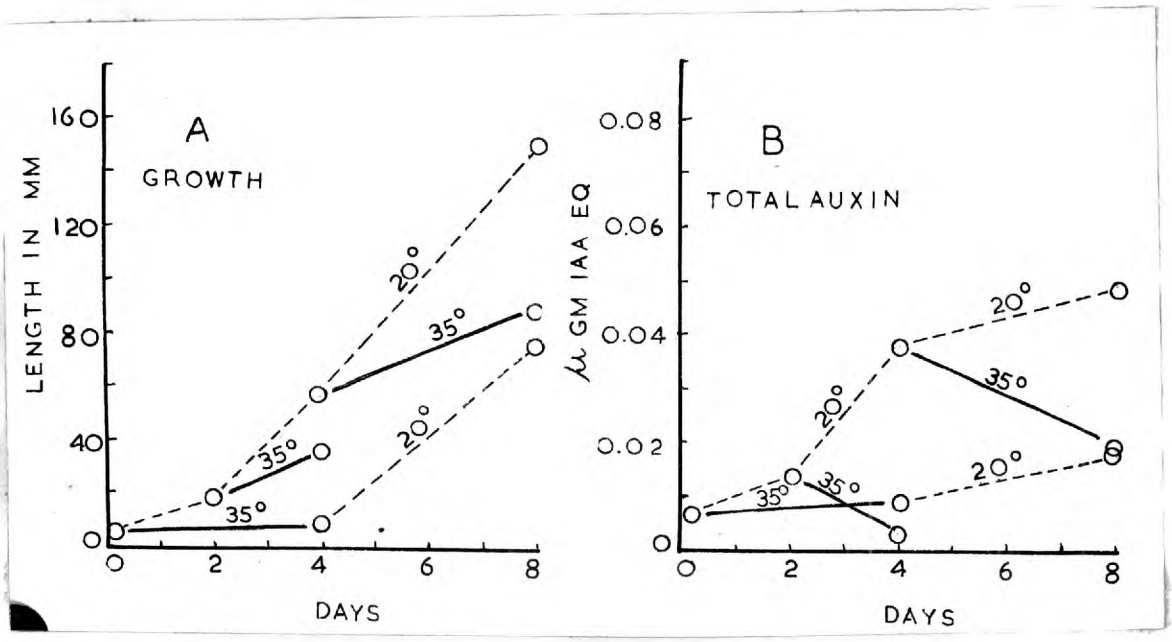

Figure 9. Effect of transference from either low to high or high to low temperature on (A) growth and (B) auxin content of excised embryos in the dark on 2% sucrose. Solid lines, embryos treated under high temperature (35°C). Broken lines, embryos treated under low temperature (20°C).

were cultured from 0 to 4 days at 35°C then were transferred to 20°C for 4 days. About 0.010 μgm of auxin was found to accumulate in embryos between the 4th and 8th day. This result shows that at high temperature the mechanism of auxin production is not completely destroyed.

3. Auxin content of excised embryos of different ages in relation to the oxygen supply.

It has been known for a long time that the growth of the embryo requires oxygen. Bonner (1933) found that cyanide acting as a respiratory inhibitor also inhibited growth. Commoner and Thimann (1941) found that iodoacetate at a concentration of 2×10^{-5} M inhibits growth completely after a few hours delay while the rate of respiration is reduced only about 10%. Similarly Bonner and Wildman (1946) found that fluoride at a low concentration which inhibits growth does not appreciably reduce the oxygen uptake. Commoner and Thimann, however, found that though coleoptile sections in water show no increased respiration when indoleacetic acid is added, ~~but~~ if the sections have been kept a few hours in sugar solution a definite rise in respiration follows almost immediately the addition of indoleacetic acid. They concluded that a respiratory enzymic reaction is an integral part of the growth process and that

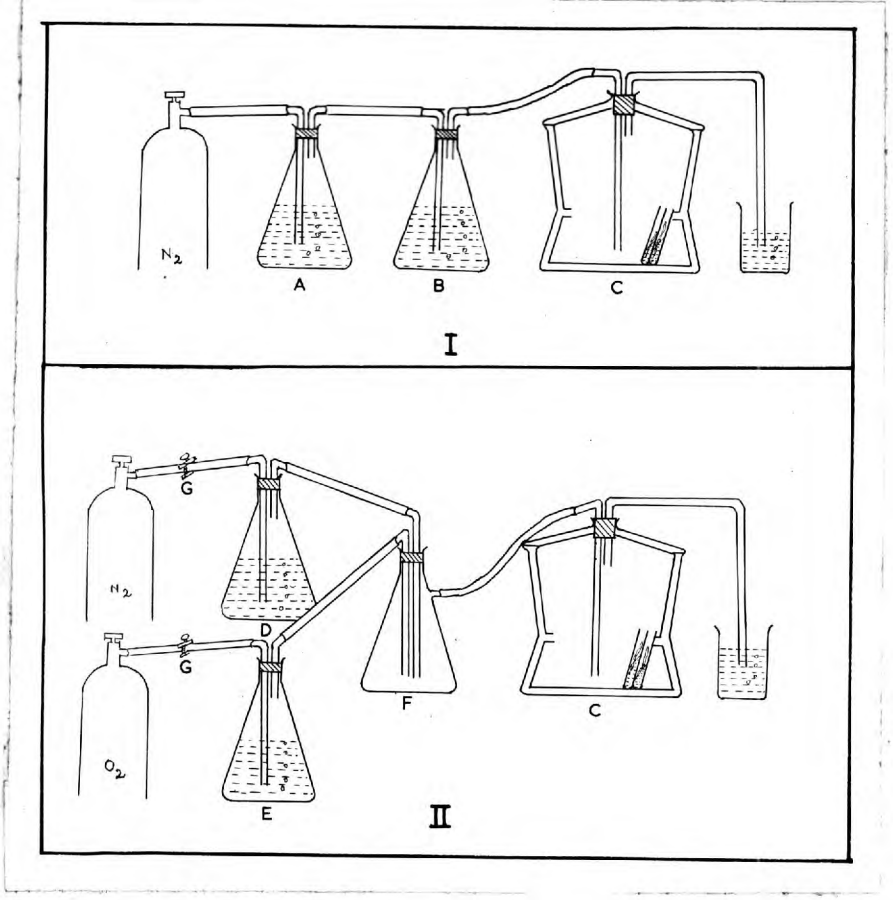

Figure 10. I. Apparatus for culturing embryos in anaerobic condition. Excised embryos were grown in test tubes in the desiccator C. Traces of oxygen from commercial nitrogen were removed by bubbling the gas through the solution containing 20% Sodium Hydrosulphite, 10% Sodium Hydroxide and 2% Anthraquinone 2-sulphonic acid ("Silver salt") in water in two conical flasks A and B.

II. Apparatus for culturing embryos in different combinations by volumes of oxygen and nitrogen. Excised embryos were grown in test tubes in the desiccator C. Volume of oxygen and nitrogen was controlled by counting the number of bubbles in the water D and E regulated by screwed pinch cocks G.

auxin in some way accelerates or acts as a Coenzyme for this reaction. Again Anker (1953) has found that the degree of stimulation of respiration by growth substances in non-growing sections of coleoptile (cut from very long coleoptiles) is not less than that in growing sections. The increase in oxygen uptake, therefore, is not the result of increased growth of the sections. In fact the part played by auxin in respiration has not been definitely established nor has the relation of oxygen uptake to the synthesis and accumulation of auxin in the plant been studied. The following experiment was carried out under conditions of controlled ~~respiration~~ aeration. Excised embryos of winter rye on 2% sucrose medium were cultured for 2, 4 and 8 days in a desiccator through which different combinations by volume of oxygen and nitrogen were passed (Figure 10); at the end of the scheduled period the native auxin content of embryos was determined by the method described on page 15-17 of this paper. The experiment was carried out at 20 °C in the dark. The results of this experiment are given in tables (14, 15) and figure (11).

Auxin content of embryos in anaerobic culture.

The growth and accumulation of auxin in embryos was almost completely inhibited in 100% nitrogen. Embryos, however, remained viable after 8 days anaerobic culture; not only could such embryos resume their growth but they could synthesize auxin after their transference from anaerobic

TABLE 14

Growth and auxin content of excised embryos of Petkus winter rye in relation to the concentration of oxygen. Auxin was extracted from duplicate samples each of 10 excised embryos in 100 cc. of water.

	Growth of Embryos				Auxin content	
Treatment	Days old	Embryo Length mm	Fresh wt. gm.	Root length test plant in % of control	Total Auxin IAA Eq. µgm per embryo	Auxin conc. IAA Eq. µgm per. gm.wt.
100 % Nitrogen	2	2.9	0.03	92.1	0.011	3.66
	"	2.8	0.03	94.1	0.007	2.33
	4	2.7	0.03	91.2	0.012	4.00
	"	2.8	0.03	91.4	0.012	4.00
	8	3.0	0.03	94.2	0.006	2.00
	"	4.5	0.06	92.4	0.011	1.83
95% Nitrogen 5% Oxygen	2	12.0	0.10	90.1	0.014	1.40
	4	14.3	0.12	86.5	0.020	1.67
	"	15.6	0.14	83.8	0.026	1.86
	8	27.9	0.20	87.7	0.017	0.85
	"	26.0	0.20	87.6	0.018	0.90
90% Nitrogen 10% Oxygen	2	20.3	0.13	90.0	0.014	1.08
	4	64.3	0.32	78.9	0.044	1.38
	8	145.5	0.57	82.9	0.029	0.51
	"	134.9	0.52	81.4	0.034	0.65
100% Oxygen	2	22.2	0.15	93.3	0.009	0.60
	4	46.3	0.22	89.8	0.014	0.64
	"	38.1	0.21	84.5	0.024	1.14
	8	122.4	0.57	81.4	0.033	0.58
	"	106.4	0.50	83.8	0.026	0.52

TABLE 15

Growth and auxin content of excised embryos of Petkus winter rye transferred (A) from air to anaerobic condition and (B) from anaerobic to aerobic condition. Auxin was extracted from duplicate samples each of 10 embryos in 100 cc. of water.

Growth of Embryos				Auxin content	
Treatment	Embryo length mm	Fresh wt. gm	Root length test plant in % of control	Total Auxin IAA Eq. µg per embryo	Auxin conc. IAA Eq. µg per gm. wt.
2 days in air then 2 days in Nitrogen	27.2	0.20	89.2	0.015	0.75
"	23.5	0.21	88.5	0.016	0.76
4 days in air then 4 days in Nitrogen	76.6	0.53	84.5	0.024	0.45
"	71.0	0.52	79.5	0.041	0.79
4 days in Nitrogen then 4 days in air	35.3	0.24	91.1	0.012	0.50
"	27.2	0.20	79.5	0.041	2.1
2 days in air	22.2	0.14	92.5	0.011	0.79
"	20.6	0.12	91.3	0.012	1.00
4 days in air	58.8	0.36	81.8	0.032	0.89
"	59.6	0.38	80.6	0.035	0.92
8 days in air	146.9	0.78	77.9	0.049	0.63

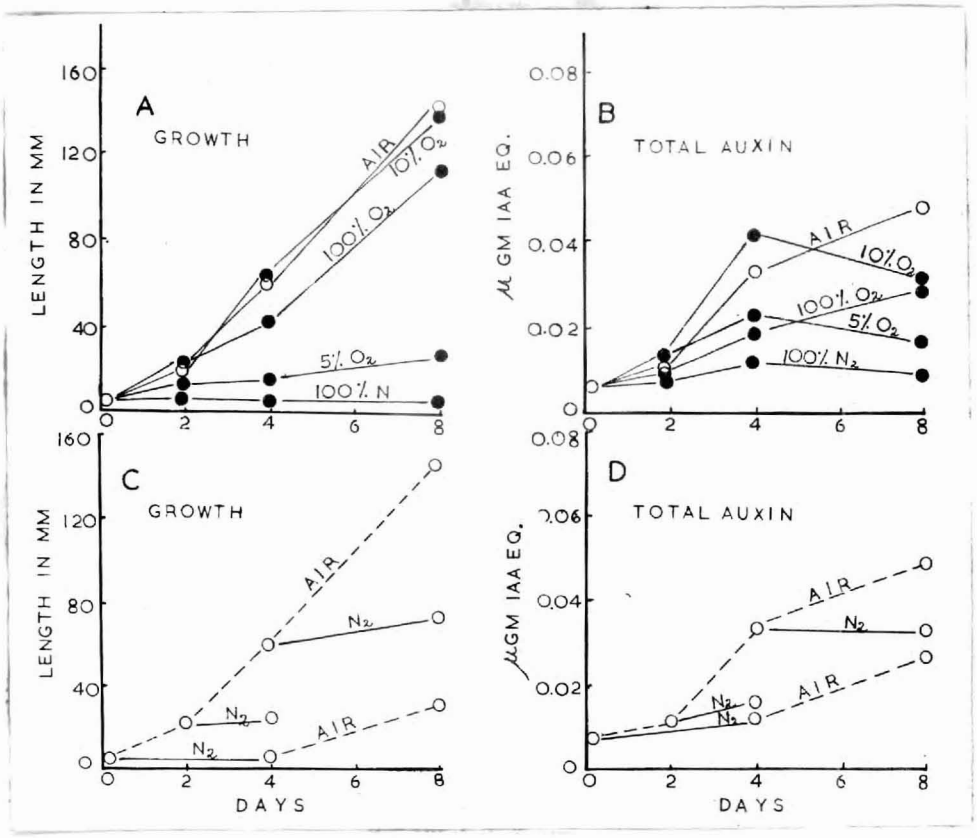

Figure 11. **A.** Growth of excised embryos in different percentages by volume of oxygen and nitrogen. Open circles, embryos growth in air.

B. The total auxin content of excised embryos cultured in different percentages by volume of oxygen and nitrogen. Open circles, total auxin of the embryo cultured in air.

C. Growth of the excised embryo in air (broken lines). Growth of the excised embryo in 100% nitrogen.

D. The total auxin content of the excised embryos cultured in air (broken lines), the total auxin content of the excised embryos in 100% nitrogen (solid lines).

condition to air; though the growth rate and production of auxin were greatly reduced by this treatment. Anaerobic culture, unlike high temperature, does not lead to the disappearance of auxin. When embryos were transferred from air to anaerobic conditions, whether after 2 days or 4 days, the level of auxin present at transfer was maintained. The sharp rise in auxin normally occurring between the 3rd and 4th days in air was inhibited by lack of oxygen, but auxin already present was not reduced. Oxygen, therefore, appears to be necessary for the production of auxin in embryos. Embryos respiring anaerobically do not accumulate auxin nor exhibit any sign of growth.

It appears also that though embryos have accumulated auxin during 4 days growth in air, no further growth is possible without oxygen ~~indicating that the effect of auxin on growth depends at some stage on air oxidative reaction~~; thus in the absence of oxygen no matter how much auxin is present it is without effect on growth.

Embryos in 5% Oxygen

In the atmosphere containing 5% oxygen and 95% nitrogen some growth and auxin accumulation takes place. In the first two days the growth amounts to some 50% of that in air, but the acceleration of growth is less so that the ~~total~~ final growth in 8 days is only 20% of that in air. During the first four days some auxin accumulated, amounting to 75% of that in air, but after fourth day there was no further auxin increase noted.

Embryos in 10% Oxygen

In the atmosphere of 10% oxygen and 90% nitrogen growth of the embryo was accelerated and total auxin also found to increase with growth in the first four days. After the fourth day no auxin has been found to accumulate in the embryo, though growth of the embryo still continued. These findings are comparable with that of embryos in suboptimal temperature or sucrose concentration.

Embryos in 100% Oxygen

In 100% oxygen shoot growth of excised embryos was inhibited about 25% but root growth was almost uninhibited. An appreciable decrease of total amount of auxin in comparison with that of embryos growing in air was also noted. The cause of reduced shoot growth and auxin accumulation in 100% oxygen is not very clear. Albaum et al (1942) suggested that 100% prevents nitrogen assimilation.

4. Auxin content of the apex, growing stem and developing ear of Petkus winter rye in relation to vernalisation and day length

In this experiment plants were grown under five different treatments. (1) Plants from six weeks vernalised seed grown under continuous light (6V. CL.) and (2) under 8 hours day length (6V. SD.). (3) Plants from unvernalised seed grown under continuous light (OV. CL.), (4) under 8 hours day length (OV.SD.) and (5) 17 hours normal summer day (OV. ND.). The auxin content of their apices, elongating stems and developing ears was estimated, and comparisons between the treatments were made when the plants attained similar stages of flower development (i.e., "scores", Table 16).

Auxin content of apices before and after flower initiation.

The apices of plants treated in these ways were dissected at the age of 15-16 days, when the apices were still undifferentiated. The auxin was extracted by ether, 10 - 20 apices were used for each 100 cc of extract and determin-ed by the method described on page 15-17 of this paper.

TABLE 16

Scoring Table

(Gregory and Purvis 1938, modified by Gott 1950)

Condition of Apex.	Score.
Double ridges just perceptible	21
" established	22
" to base of spike	23
Spikelet ridges swelling	24 - 25
" branching	26 - 27
Flower initials appear	28 - 29
Stamen initials appear	30 - 31
" lobed	32 - 33
Awns begin growth	34
Awns growing barbed, ear still stiff	35
Awns long, ear limp	36
Flag leaf emerged, ear limp	37
" ear stiff, green	38
Early earing 1, (tip of awns just visible above flag leaf)	39
" (awns emerging)	40
Early earing 2, (awns completely emerged)	41
Earing 1, (less than $\frac{1}{2}$ of ear emerged)	42
Earing 2, (more than $\frac{1}{2}$ of ear emerged)	43
late earing 1, (peduncle just visible)	44
" 2 to 5, (peduncle emerging to full length)	45 - 48
Anthesis	49

Figure 12. Photograph of undifferentiated apices of Petkus winter rye,(Score below 21).

Figure 13. Photograph of apices of Petkus winter rye during floral initiation. ~~From left~~ : 3,4,8,9,10,11, floral initiation has just occurred(score 21-23). 5, ridges swelling at the tip (score 24-25); 2, flower initials being formed (score 28-29); 1, stamen initials being formed (score 30-31).

Figure 14. Photograph of developing ears of Petkus winter rye. From left: 5, stamen initials lobed (score 32-33); 4,3,2, awns begin to grow (score 34); 1, the grand period of elongation of ear has started (score 35).

Figure 16.

Figure 15.

Figure 17.

Figure 15. Photograph of developing ears of Petkus winter rye. At the end of grand period of elongation, pedancle just beginning extension (score 36-37).

Figure 16. Ear emergence. Left : score 39-40; right : score 42-43.

Figure 17. Anthesis of the ear (score 49).

The concentration of auxin in these undifferentiated apices was found to be high (Table 17, 18, 19) and figure (18). The concentration of auxin in apices was on the whole higher under short days than under continuous light, but this difference was not consistent. The total quantity of auxin of these small undifferentiated apices varied from 0.002 μgm to 0.006 μgm according to treatment and was the lowest content found.

Petkus winter rye being a facultative long day plant the differentiation of double ridges (score 21-23) was attained at very different ages according to treatment, e.g., in 6V. CL., the plants reached this stage at the age of 22 days, in 0V. CL., in 36 days and in 6V. SD., in 84 days. The auxin content of these apices immediately after differentiation of double ridges was estimated and compared. Although their ages were widely different the auxin content of these apices was approximately the same. No consistent change in auxin content accompanied the first stage of flower differentiation (double ridges). These concentrations appeared to be very high and differed greatly from those obtained when the differentiated apices started to elongate and underwent further development.

TABLE 17

Auxin content of apices, developing ears and extending stems of Petkus winter rye, vernalised for six weeks and grown under continuous light. Ether extraction. Dilution was 100 cc from "scores" below 36, and 1000 cc for higher "scores" (36 to 49).

6V. CL.	Score	Days old	Length cm	Fresh wt. gm.	R.T.P. %	Total Auxin IAA Eq. μ gm per Apex, Stem or ear	Auxin conc IAA Eq. μ gm per gm. wt.
20 APICES							
Simple ridges	Below 21	15	0.03	0.0012	96.0	0.0016	26.66
	"	"		0.0014	94.8	0.0025	35.71
Double ridges	21-23	22	0.16	0.0017	90.7	0.0063	74.11
	"	23	0.18	0.0017	93.0	0.0050	58.82
10 STEMS							
Young stem	34-35	36	4.1	1.43	58.3	0.36	2.52
	"	"	3.9	1.40	59.4	0.31	2.21
Flag leaf emerged	36-37	39	22.3	7.93	69.2	1.18	1.49
	"	"	24.2	8.13	72.9	0.81	0.99
Early earing	39-40	42	35.0	10.12	72.0	0.87	0.86
	"	43	35.6	11.96	73.9	0.72	0.60
Anthesis	49	58	78.0	22.40	74.9	0.66	0.29
	"	"	76.7	23.25	74.2	0.69	0.30
10 EARS							
Stamen developing	30-33	29	0.4	0.015	91.8	0.01	6.67
	"	30	0.5	0.014	90.0	0.01	7.14
Rapid growth of ear	34-35	36	2.0	0.11	85.3	0.02	1.82
	"	"	1.8	0.12	86.1	0.02	1.67
Flag leaf emerged	36-37	39	4.9	1.52	92.4	0.11	0.72
	"	"	5.0	1.60	92.0	0.11	0.69
Early earing	39-40	42	5.0	2.51	67.5	1.38	5.49
	"	43	5.3	2.64	72.0	0.87	3.29
Anthesis	49	58	7.5	6.23	64.3	1.95	3.13
	"	"	7.6	6.54	66.0	1.62	2.48

R.T.P. Root length of test plant in percentage of control.

TABLE 18

Auxin content of apices, developing ears and extending stems of unvernalised Petkus winter rye grown under continuous light. ETher extraction. Dilution was 100 cc from "scores" below 36, and 1000 cc for higher "scores" (36 to 49).

OV. CL.	Score	Days old	Length cm	Fresh wt. gm	R.T.P. %	Total Auxin IAA Eq. µ gm per Apex, Stem or Ear	Auxin conc. IAA Eq. µ gm per gm.wt.
20 APICES							
Simple ridges	below 21	15	0.02	0.0010	92.0	0.0056	112.00
	"	"		0.0015	94.8	0.0025	33.33
Double ridged	21-23	36	0.19	0.0012	90.8	0.0063	105.00
	"	37		0.0018	92.5	0.0053	58.88
10 STEMS							
Young stem	34-35	53	4.0	1.88	58.3	0.36	1.91
	"	"	4.1	1.84	63.5	0.21	1.14
Flag leaf emerged	36-37	72	23.0	12.20	72.8	0.81	0.66
	"	"	22.1	11.78	69.5	1.12	0.95
Early earing	39-40	103	66.0	28.23	73.8	0.72	0.26
	"	"	64.0	26.52	72.5	0.83	0.31
Anthesis	49	120	104.0	32.12	76.8	0.54	0.17
	"	"	99.0	34.34	75.8	0.59	0.17
10 EARS							
Stamen developing	30-33	46	0.5	0.016	90.8	0.01	6.25
	"	47	0.5	0.015	92.5	0.01	6.66
Rapid growth of ear	34-35	53	1.7	0.12	84.0	0.03	2.50
	"	"	1.6	0.13	86.0	0.02	1.54
Flag leaf emerged	36-37	72	6.7	1.20	87.8	0.17	1.42
	"	"	6.9	1.42	89.0	0.15	1.06
Early earing	39-40	103	8.1	8.14	68.3	1.29	1.58
	"	"	7.6	7.52	69.0	1.20	1.60
Anthesis	49	120	8.8	8.24	64.3	1.95	2.37
	"	"	9.0	9.32	60.3	2.95	3.17

R.T.P. Root length of test plant in percentage of control.

TABLE 19

Auxin content of apices before and after floral initiation in Petkus winter rye; (1) unvernalised seeds grown under short days (8hr. days); (2) 6 weeks vernalised seeds grown under short days (8hr. days); (3) unvernalised seeds grown under normal summer days. Ether extraction. Dilution 100 cc.

Treatment	Score	Days old	Length in mm.	Fresh wt. gm.	R.T.P. %	Total Auxin IAA Eq. µgm per apex	Auxin conc IAA Eq. µgm p r gm.w
(1)							
20 Apices O.V. S.D.	Below 21	15	0.17	0.0009	91.8	0.006	133.3
" "	"	"		0.0010	95.7	0.002	30.0
20 Apices OV. SD.	21-23	140	1.5	0.0015	93.8	0.003	46.6
" "	"	"		0.0010	98.4	0	0
(2)							
20 apices 6V.SD.	Below 21	15	0.18	0.0007	94.2	0.003	85.7
" "	"	"	0.16	0.0008	91.7	0.006	137.5
20 Apices 6V. SD.	21-23	84	1.2	0.0012	93.0	0.005	83.3
" "	"	"		0.0010	92.0	0.006	120.0
(3)							
20 Apices OV. ND.	Below 21	17	0.25	0.0015	93.5	0.004	53.3
" "	"	"		0.0012	93.8	0.004	86.6
20 Apices OV. ND.	21-23	96	1.0	0.0018	93.7	0.004	44.4
" "	"	"		0.0017	90.0	0.007	82.3
10 Ears OV. ND.	34-35	139	5.0	0.14	90.8	0.012	0.9
10 Stems OV.N.D.	34-35	139	112	5.8	53.0	0.616	1.1
" "	"	"	90	5.0	55.0	0.513	1.0

R.T.P. Root length of test plants in percentage of control.

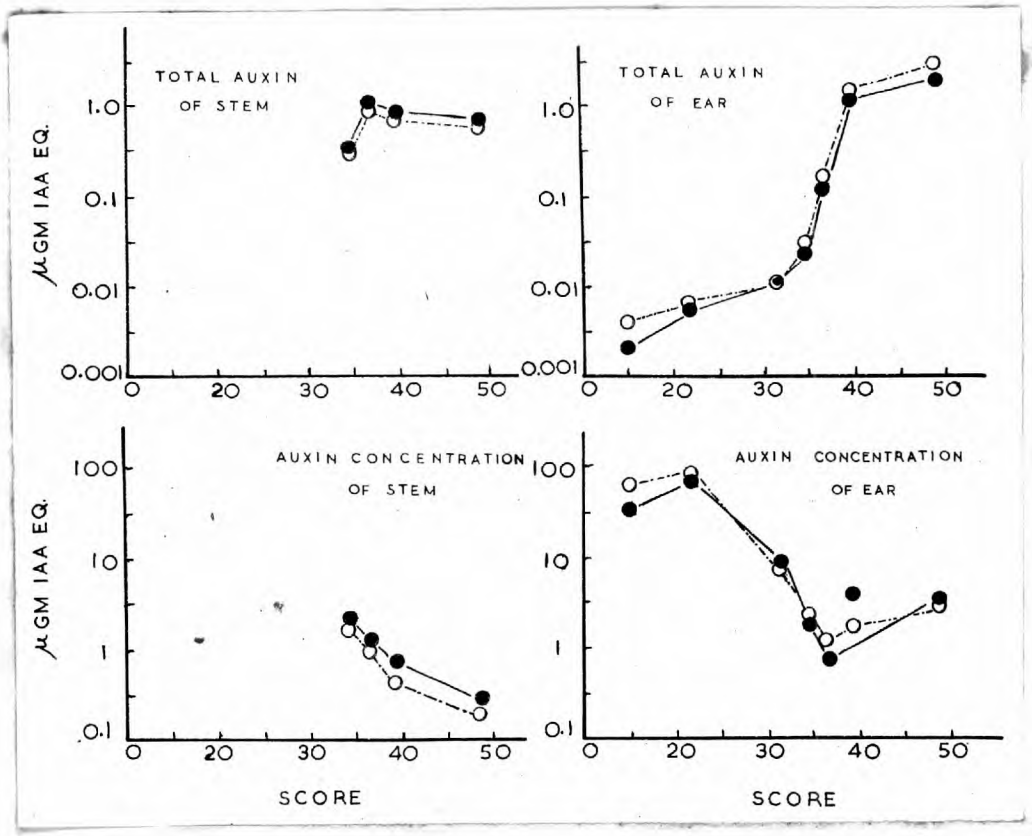

Figure 18. Auxin content of the extending stem and developing ear of Petkus winter rye in relation to development of apex ("Score"). Closed circles, six weeks vernalised plants grown under continuous light (6V.CL.). Open circles, Unvernalised plants grown under continuous light (0V.CL.).

Auxin content of stems during extension growth

In the developing stem the concentration of auxin varies only with the score. The stem started to elongate when the score was 30-32. In all treatments the concentration of auxin in the stem was found to decrease very rapidly with increasing score. The decrease in concentration was approximately inversely proportional to the stem elongation and, therefore, showed approximate linear relationship (Figure 19) with extension growth right up to the stage of anthesis. In the 6V. CL. series as the "score" rose from 34-49 the concentration of auxin decreased from 2.4 µgm to 0.30 µgm per gm.wt. of stem, ~~when~~ while stem length had increased from 4.0 cm to 77.3 cm and the corresponding weight increment was found to be from 0.14 gm to 2.3 gm per stem. Similarly in the 0V. CL. treatment the concentration of auxin decreased from 1.5 µgm to 0.17 µgm per gm.wt. of the stem ~~when~~ while the stem attained the size 102 cm from 4.0 cm ~~to 102 cm~~.

The level of the total quantity of auxin in the stem, on the other hand, ~~was~~ increased with the score up to the stage of ear emergence (score 39-40); after that stage although stem length ~~was~~ continued to increase the total quantity of auxin maintained a steady level. At higher scores the total auxin was sometimes less than

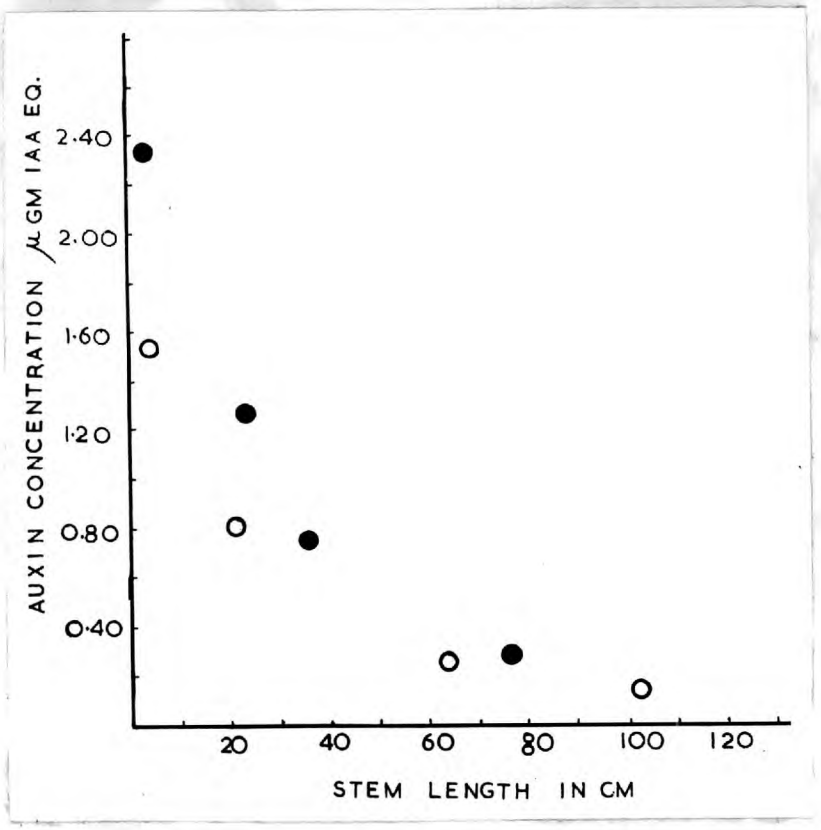

Figure 19. Relation of concentration of auxin in the stem to extension growth. Closed circles, six weeks vernalised plants grown under continuous light (6V.CL.). Open circles, unvernalised plants grown under continuous light (0V.CL.).

that of plants at score 39-40.

Auxin content in developing ears

In the developing ears the concentration of auxin again depends on the "score" and not on the "treatment". The total quantity of auxin in the treatment OV. CL., was slightly higher than that of 6V. CL., this, however, was probably related to the greater growth of the ear in OV. CL.

The concentration of auxin in all the treatments was at first found to decrease steadily with score as it did in the stem. As the score rose from 21-23 to 36-37 the average auxin concentration in ear fell from its peak value of about 73.0 μgm to 0.98 μgm. During this phase the ear completed most of its growth in length from 0.05 cm to 7 cm. At the score of 39-40, when the ear had just emerged and turned green and was approaching its maximal length a rise in the concentration of auxin was found again in the ear. Auxin content per gm. wt. of the ear was found to increase with score up to anthesis. This period of increasing concentration coincided with the formation of pollen grains and ovules. (Figure 15, 16, 17).

Although the concentration of auxin shows a fall and later a rise, the total quantity of auxin in the ear steadily increased after differentiation of the apices up to anthesis, (Figure18).

5. Auxin content of the apex, extending stem and the developing ear of Petkus winter rye transferred (A) from long days to short days and (B) from short days to long days.

The significant facts that have been gathered from the foregoing experiment are that irrespective of different treatments, the amount of auxin was approximately the same in all plants at the same stage of floral development ("Score"), and the auxin balance of a plant was quite independent of its age on a time scale. Thus it appears that the auxin balance of a plant is controlled by photoperiod and temperature in the same way as growth and development.

With a view to investigate these facts more critically a series of transfer experiment was carried out. In one set of experimental plants from six weeks vernalised seeds were grown under constant 17 hours day length (normal days supplemented by artificial light) for six weeks then were transferred to 8 hours day length (6V.LD.→ SD.).

Some plants were kept in 17 hours day length throughout the period of experiment as control (6V. LD.). In other set of experiment, plants from similarly treated seeds were grown under 8 hours day length for six weeks and were then transferred to days of constant 17 hours length (6V. SD. → LD.). Some plants were kept in 8 hours day length throughout as control (6V. SD.).

Plants were dissected out at the age of 17 and 19 days; under both day length the apices were at this time below the score of 21. The quantity and concentration of auxin was found a little higher in apices of plants in short days than that of plants in long days (Table 20) not significant Similar result was obtained in previous experiment where apices of plants in short days contained more auxin and at a higher concentration than those in continuous light. The plants under long-day treatment reached the stage of double ridges in just under 5 weeks. On the day of transference the plants in long days reached the score 28-31, a few of them were 33-34 with visible external sign of stem extension; in these stages they were transferred to short days. The "score" of plants which were transferred from short days to long days (6V. SD. → LD.), was still below 21.

TABLE 20

Effect of day length on auxin content of apices before and after floral initiation in Petkus winter rye vernalised for six weeks. Ether extraction. Dilution 100 cc.

Treatment	Score	Days old	length cm	Fresh wt. gm	R.T.P. %	Total Auxin IAA Eq. µgm per apex	Auxin conc. IAA Eq. µgm per gm. wt.
40 APICES							
6V. LD.	Below 21	17	0.03	0.0016	94.6	0.0014	35.00
"	"	"		0.0020	92.9	0.0026	52.00
6V. SD.	Below 21	19	0.02	0.0017	91.9	0.0029	68.24
"	"	"		0.0013	91.5	0.0029	89.23
20 APICES							
6V. LD.	21-23	32	0.20	0.0020	91.0	0.0062	62.00
"	"	"		0.0015	92.5	0.0059	78.67
6V. SD.	21-23	77	0.15	0.0015	95.7	0.0017	22.67
"	"	"		0.0015	93.0	0.0050	66.67
6V.SD→LD	21-23	48	0.18	0.0018	92.5	0.0052	57.78
"	"	"		0.0020	90.0	0.0069	69.00

R.T.P. Root length of test plant in percentage of control.

TABLE 21

Auxin content of extending stems of Petkus winter rye vernalised for six weeks. Plants were transferred (A) long days to short (6V. LD.+SD) or from (B) short days to long days (6V. SD.+LD). Auxin was extracted from each sample of 10 stems. Ether extraction. Final dilution, 1000cc

Treatment	Score	Days old	Length cm	Fresh wt. gm	R.T.P. %	Total Auxin IAA Eq. µgm per stem	Auxin conc. IAA Eq. µgm per gm. wt.
6 V. LD.	34-35	47	4.8	1.62	83.4	0.28	1.73
"	"	"	5.0	1.82	81.4	0.34	1.87
6V.LD.+SD.	34-35	53	5.5	1.40	89.7	0.14	1.00
"	"	"	6.5	2.00	87.3	0.18	0.90
6V.SD.+LD.	34-35	60	4.7	1.80	83.3	0.28	1.56
"	"	"	4.5	1.70	82.6	0.30	1.76
6V. LD.	36-37	49	13.3	5.02	79.6	0.41	0.82
"	"	"	12.6	6.00	73.3	0.78	1.30
6V.LD.+SD.	36-37	58	12.0	3.00	94.6	0.06	0.20
6V.SD.+LD.	36-37	64	13.3	4.38	77.1	0.53	1.21
6V. LD.	40-41	53	43.1	13.56	71.5	0.93	0.69
"	"	"	42.0	11.00	78.7	0.44	0.40
6V.LD.+SD.	40-41	60	16.4	4.16	83.6	0.27	0.65
6V.SD.+ LD.	40-41	78	39.2	11.44	73.4	0.78	0.68
"	"	"	40.0	12.00	74.6	0.68	0.57
6V. LD.	49	62	66.0	13.36	77.4	0.51	0.38
"	"	"	70.0	16.2	74.5	0.68	0.42
6V.LD.+SD.	49	64	31.5	7 .30	82.3	0.30	0.41
"	"	"	"	"	82.5	0.30	0.41
6V. SD.+ LD.	49	81	71.2	16.6	71.4	0.96	0.58
"	"	"	69.5	13.5	80.2	0.37	0.27

R.T.P. Root length of test plant in percentage of control.

TABLE 22

Auxin content of developing ears of Petkus winter rye vernalised for six weeks. Plants were transferred from (A) long days to short days (6V. LD. → SD.) or from (B) short days to long days (6V. SD. → LD). Auxin was extracted from each sample of 10 ears. Ether extraction.

Treatment	Score	Days old	length cm	Dilution	Fresh wt. gm	R.T.P. %	Total Auxin IAA Eq. µgm per ear	Auxin conc. IAA Eq. µgm per gm. wt.
6V. LD.	30-31	41	0.4	0.1	0.007	94.1	0.0068	9.71
"	"	"	0.3	"	0.006	94.6	0.0055	9.17
6V. LD.→SD.	30-31	41	0.3	0.1	0.010	94.7	0.0050	5.00
"	"	"	0.3	"	0.009	92.5	0.0105	11.66
6V. SD.→LD.	30-31	48	0.3	0.1	0.008	93.3	0.0091	11.37
"	"	"	0.4	"	0.010	91.8	0.0115	11.50
6V. LD.	34-35	47	0.9	0.1	0.055	87.5	0.0178	3.24
6V. LD.→SD.	34-35	53	0.7	0.1	0.050	91.4	0.0120	2.40
6V. SD.→LD.	34-35	58	0.8	0.1	0.050	88.5	0.0159	3.18
6V. LD.	36-37	49	4.3	1.0	1.20	91.4	0.120	1.00
"	"	"	5.0	"	1.50	87.8	0.174	1.16
6V. LD.→SD.	36-37	58	3.8	1.0	0.63	95.4	0.042	0.67
"	"	"	3.5	"	0.68	94.0	0.068	1.00
6V. SD.→LD.	36-37	64	5.0	1.0	1.50	89.9	0.141	1.19
6V. LD.	40-41	53	6.6	1.0	5.58	73.6	0.76	1.36
"	"	"	6.8	"	5.80	69.1	1.20	2.07
6V. LD.→SD.	40-41	60	4.1	1.0	2.00	88.1	0.17	0.85
6V. SD.→LD.	40-41	78	7.2	1.0	6.56	65.7	1.66	2.53
6V. LD.	49	62	7.6	1.0	6.72	60.0	3.02	4.49
"	"	"	6.0	"	5.00	64.3	1.00	2.00
6V. LD.→SD.	49	64	5.0	1.0	2.50	91.2	0.12	0.48
"	"	"	"	"	"	79.3	0.42	1.68
6V. SD.→LD.	49	81	6.5	1.0	5.60	60.2	2.95	4.24
"	"	"	7.2	"	6.40	62.8	2.29	3.56

R.T.P. Root length of test plants in percentage of control.

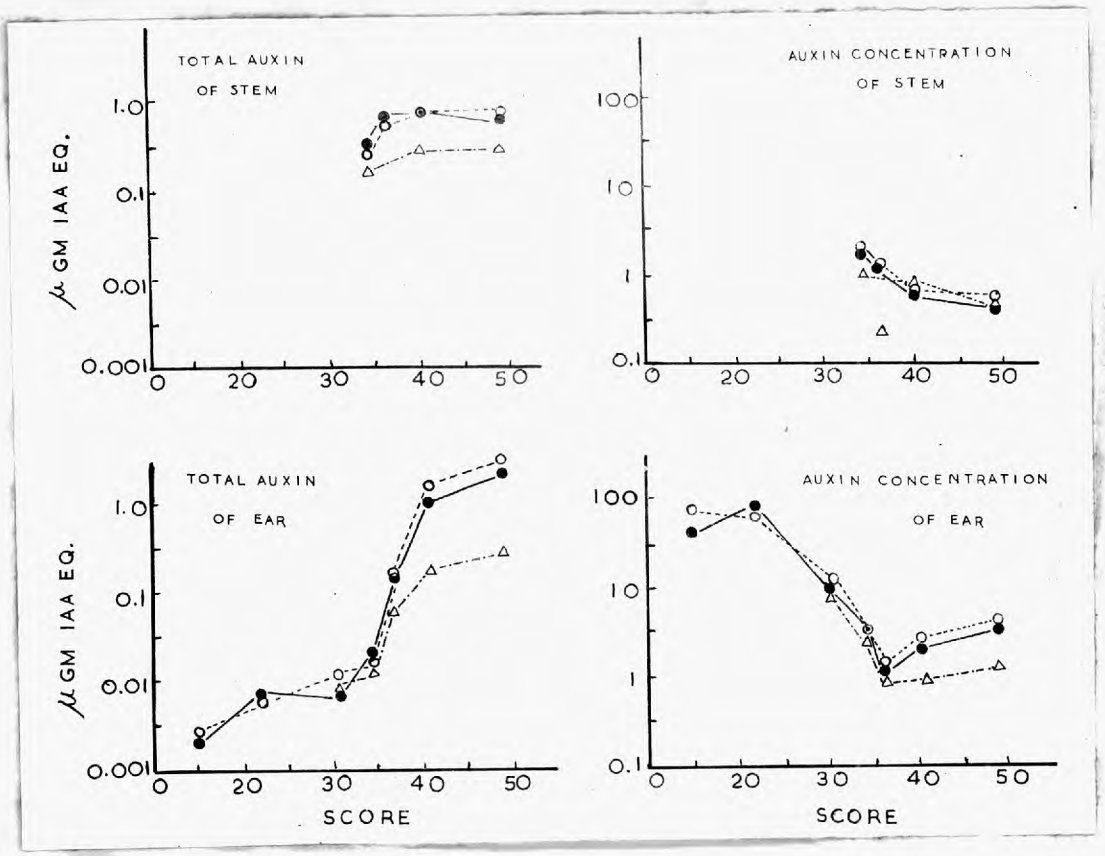

Figure 20. Auxin content of the extending stem and developing ear of vernalised Petkus winter rye in relation to 'score' under the treatments of different day length. Closed circles, plants under constant long days of 17 hours (6V.LD.). Open circles, plants given six weeks short days (8 hours) then transferred to long days (6V.SD-LD.). Triangles, plants given six weeks long days then transferred to short days (6V.LD-SD).

Auxin content of plants transferred from long days to short days (6V. L.D. → SD.)

From the table (21, 22), and figure (20), it can be seen that neither the auxin accumulation nor the stem elongation of plants stopped abruptly when they were transferred from long days to short days, though the rate of auxin accumulation and stem elongation was found to decrease in comparison with their 17 hours long day control. The plants under 6V. LD. - SD., reached the score 34 - 35 at the age of 53 days - 6 days later than their control. About that stage approximately 60% of plants stopped their further stem extension and ear development but 40% of them passed the score 36-37 and 40-41 under short day condition with reduced size of ear and stem height. Some of these plants were able to reach the stage of anthesis about the same time with same leaf number as did the controls in 17 hours long days. The extension growth of the stem and development of the ear were greatly ~~inhibited~~ reduced under short day condition, only a few of the carpels and anthers became fertile at the stage of anthesis and the rest of them remained in a sterile condition and peduncle was so much reduced in ~~height~~ length that the ear did not fully emerge from the last leaf.

The length of stem and ear being shorter the total quantity of auxin, was much less than in the controls (17 hour day), but the concentration of auxin in stem did not differ from the control. The lower concentration of auxin in the ear may be, however, due to the lack of normal development of the carpel and anther under short day condition leading to a semisterile state during the time of anthesis.

A similar result was also obtained by Gott (Ph.D. thesis L.U. 1950). She transferred unvernalised winter rye from continuous light to short days. Before transfer plants ~~were~~ had received 2, 3, 5, 7, or 9 weeks of continuous light. Of the plants given 2, 3, 5, or 7 weeks of continuous light none ~~of them~~ were able to reach the stage of anthesis nor did the extension growth of stem occurred under short days. But plants which received 9 weeks continuous light ~~and~~ of which 25% to 50% ~~of which~~ showed the visible external sign of stem extension (score 33-34) before transfer, some ~~of them~~ did reach ~~the~~ anthesis under short days, but with reduced size of ear and stem height. It is, therefore, suggested that the high concentration of auxin in the stem of rye associated ~~accumulated~~ at the score 33-34, which in rye preceeds the grand period of ear and stem growth, marks a critical stage in development, beyond which the ability to complete ear formation and stem extension is established.

Auxin content of plants transferred from short days to long days (6V. SD. → LD)

The auxin content in stem and ear of plants transferred from short days to long days closely followed the course of long day control, though they ~~were~~ differed widely ~~apart~~ from each other regarding their age. At the age of six weeks they were transferred from short days to long days; apices were still undifferentiated at that time, but within a week they formed double ridges. The total auxin content of the stem increased during growth and ear development, up to ear emergence. Auxin formation, however, did not keep pace with stem growth, and the concentration fell rapidly throughout the period of extension up to anthesis.

In the developing ear the concentration of auxin was at first found to decrease steadily with "score", during this phase the ear completed most of its growth in length; when the ear had emerged and turned green, a rise in the concentration of auxin was found again in the ear this phase coincided with the formation of ovules and pollen grains. On the other hand the total quantity of auxin in the ear steadily increased after differentiation of the apices up to anthesis. The amount of auxin found at different stages of development of stem and ears was approximately the same as that found at corresponding stages of the long day control.

These results are, therefore, in agreement with the findings of previous experiment.

6. Auxin content of nodes and internodes of Petkus winter rye in relation to the extension growth.

The extension growth of the stem of rye starts at about the time stamen initials are being formed and continues until shortly after anthesis. In most cases extension begins in the 4th internode from the top; higher internodes extend in sequence towards the apex (acropetal succession), each successive internode reaching a greater final length. The figure (21) shows the order of extension and degree of elongation of internodes at different stages of floral development (denoted by scores). The peduncle starts to extend before ear emergence and during the stage of anthesis it attains a greater length than any other internode. After six weeks vernalisation, rye forms 10-11 leaves before flowering indicating the presence of 9-10 internodes in the body of the crown but only the upper 4 or 5 of them extend and the older ones remain compressed. In the account which follows the internodes are numbered from the apex downwards.

During the grand period of growth in the developing ear (score 36-37), extension growth of all

the internodes of the stem was in progress. Auxin content of each internode was determined separately; during dissection internodes were separated ~~from~~ at the middle of nodes, so that each internode possessed a portion of nodal tissue at either end, and the sum of the auxin content of these internodes will give the total auxin content of the stem. The amount of auxin was determined following the usual method as described on page 15-17 of this paper. The results have been summarised in tables (23, 24, 25, 26) and figure (21). The following facts are apparent: the upper internodes which are beginning to extend contain a much higher concentration of auxin, though less in total quantity, than lower internodes which have already been extended. This relation was well found at score 37 and 41 but at score 49 (during anthesis), when the whole stem approached maximal extension, the auxin was found to be distributed almost uniformly throughout the stem.

As regards the total quantity of auxin there was indication of accumulation of auxin in the first and second internode during extension growth, and on the cessation of extension growth the total quantity of auxin also decreased in some cases, for example 4th and 5th internode at the time of anthesis.

TABLE 23

Auxin content of internodes of Petkus winter rye before ear emergence (score 37, age 42 days). 6 weeks vernalised seeds were grown under 17 hours constant day length.

Internodes score 37	Length cm	Fresh wt. gm	Extraction 100 cc	R.T.P. %	Total Auxin IAA Eq. µgm per internode	Auxin conc. IAA Eq. µgm per gm.wt.
1st Internode						
from top	0.3	0.009	water	93.1	0.10	11.11
"	0.4	0.007	"	92.7	0.10	14.28
"	0.3	0.012	Ether	91.6	0.12	12.00
"	0.3	0.012	"	90.9	0.13	10.83
2nd Internode						
from top	0.5	0.010	water	93.0	0.10	10.00
"	0.7	0.017	"	91.7	0.12	7.00
"	0.6	0.020	Ether	93.1	0.10	5.00
"	0.7	0.015	"	93.0	0.10	6.66
3rd Internode						
from top	4.3	0.090	water	83.0	0.28	3.11
"	3.6	0.080	"	85.9	0.21	2.63
"	3.2	0.075	Ether	87.6	0.18	2.40
"	5.4	0.145	"	80.0	0.38	2.62
4th Internode						
from top	5.0	0.098	water	80.7	0.36	3.67
"	4.3	0.115	"	80.1	0.38	3.30
"	4.7	0.122	Ether	81.5	0.33	2.70
"	5.0	0.190	"	80.1	0.38	2.00
5th Internode						
from top	1.0	0.130	Ether	84.0	0.25	1.92
"	1.0	0.135	"	83.6	0.30	2.22

R.T.P. Root length of test plant in percentage of control.

TABLE 24

Auxin content of internodes of Petkus winter rye after ear emergence (score 41, age 45 days). 6 weeks vernalised seeds were grown under 17 hours constant day length.

Internodes score 41	Length cm	Fresh wt. gm.	Extraction 100 cc	R.T.F. %	Total Auxin IAA Eq. µgm per Internode	Auxin conc. IAA Eq. µgm per gm.wt.
Peduncle	6.5	0.045	Water	93.3	0.09	2.00
"	6.3	0.055	"	95.8	0.04	0.73
"	5.3	0.035	Ether	91.7	0.12	3.43
"	7.1	0.060	"	89.9	0.14	2.33
1st Internode from top	4.8	0.050	Water	81.6	0.33	6.60
"	1.6	0.055	"	86.4	0.20	3.64
"	1.7	0.055	Ether	88.3	0.16	2.91
"	2.2	0.060	"	85.1	0.22	3.67
2nd Internode from top	7.5	0.105	Water	82.3	0.30	2.86
"	7.6	0.205	"	76.2	0.56	2.73
"	7.6	0.260	Ether	82.8	0.29	1.12
"	7.3	0.240	"	81.2	0.34	1.42
3rd Internode from top	5.0	0.140	Water	85.5	0.22	1.57
"	6.3	0.130	"	80.8	0.36	2.77
"	4.9	0.240	Ether	88.2	0.16	0.66
"	5.0	0.205	"	80.9	0.36	1.76
4th Internode from top	4.0	0.135	Ether	88.8	0.15	1.11
"	3.5	0.145	"	92.9	0.10	0.69
5th Internode from top	1.2	0.045	Water	88.1	0.16	3.55
"	1.0	0.070	"	92.2	0.10	1.43
"	1.5	0.085	Ether	93.0	0.10	1.17
"	1.4	0.080	"	95.8	0.04	0.50

TABLE 25

Auxin content of internodes of Petkus winter rye during anthesis (score 49, age 60 days). 6 weeks vernalised seeds were grown under 17 hours constant day length.

Internodes score 49	Length cm	Fresh wt. gm	Extraction 100 cc	R.T.F. %	Total Auxin IAA Eq. µgm per internode	Auxin conc. IAA Eq. µgm per gm.wt.
Peduncle	21.5	0.25	Water	85.6	0.21	0.84
"	20.0	0.24	"	84.6	0.24	1.00
"	18.0	0.22	Ether	86.8	0.19	0.86
"	19.0	0.23	"	86.3	0.20	0.87
1st Internode from top	16.4	0.46	Water	80.0	0.38	0.83
"	15.2	0.44	"	83.7	0.26	0.59
"	15.0	0.39	Ether	84.2	0.25	0.64
"	14.0	0.37	"	84.8	0.23	0.62
2nd Internode from top	12.8	0.50	Water	74.7	0.66	1.32
"	12.5	0.50	"	78.8	0.44	0.88
"	11.0	0.43	Ether	82.2	0.30	0.70
"	12.2	0.45	"	80.8	0.36	0.80
3rd Internode from top	11.3	0.35	Water	88.7	0.16	0.46
"	12.0	0.35	"	84.2	0.25	0.71
"	9.8	0.36	Ether	85.7	0.22	0.61
"	10.2	0.34	"	83.5	0.27	0.79
4th Internode from top	3.8	0.20	Water	88.1	0.17	0.85
"	4.3	0.20	"	87.6	0.17	0.85
"	6.4	0.27	Ether	85.2	0.23	0.85
"	6.0	0.26	"	89.8	0.14	0.54
5th Internode from top	1.9	0.09	Ether	99.0	0	0
"	1.8	0.08	"	98.3	0	0
"	1.2	0.06	Water	95.76	0.037	0.61

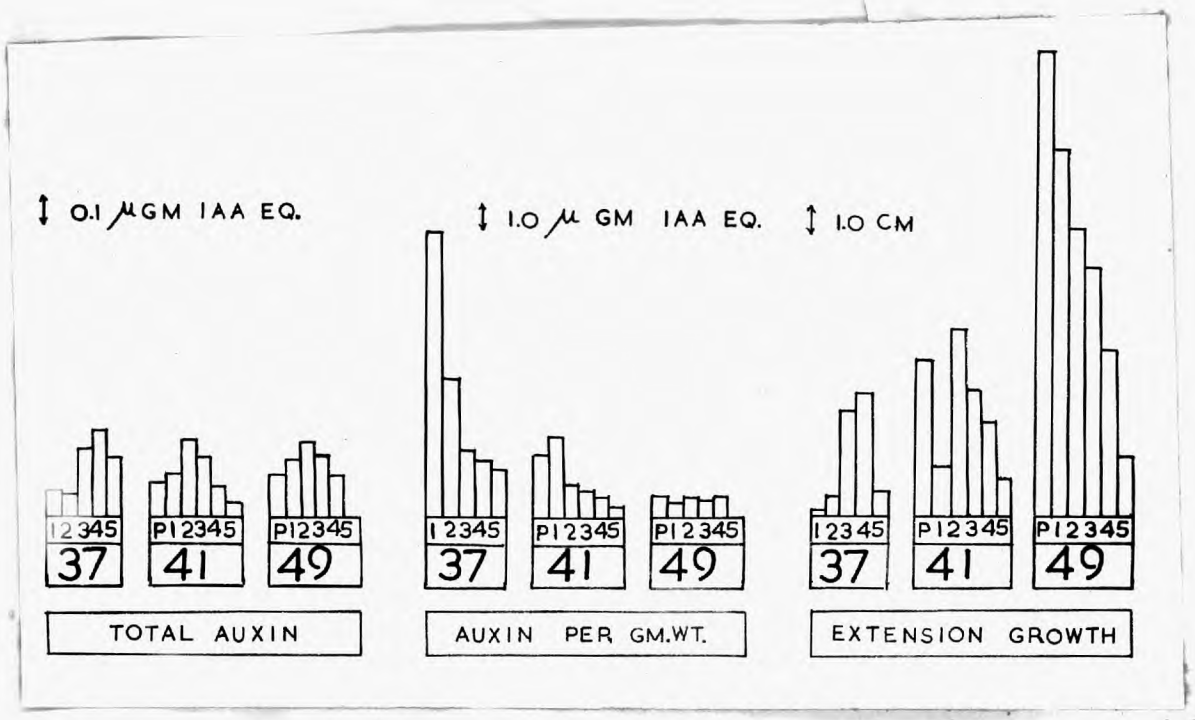

Figure 21. Growth and auxin content of different internodes of Petkus winter rye. P=peduncle.1,2,3, e.t.c.,successive internodes below peduncle.Figures below each group are'score'at the time of estimation. The figure has been drawn from the mean value of eluat extractable auxin.

The concentration of auxin in all cases was found to decrease rapidly with flower development at the apex and with growth of internodes. From "Score" 37 to 49 the concentration of auxin decreased in the first internode from 12.0 µgm to 0.64 µgm per gm. wt of tissue ~~when~~ while length had increased from 0.3 cm to 14.5 cm.

A further series of estimations was made in order to obtain the relation of auxin content between nodes and internodes. Equal weights of nodal and internodal tissue from both young (score 37) and mature (score 47) stem were taken and assayed for auxin activity. It was found that young nodes contained the highest concentration of auxin which was approximately 2.5 times higher than that of corresponding internodes. As the stem approached its maximal extension not only the absolute concentration but also the difference between the concentrations of auxin in node and internode decreased. Mature nodes (score 47) contained approximately one quarter of the auxin present in the same node at younger stages. These facts lend support to the assumption that the meristem of the node of the cereal stem is the seat of formation of auxin; hence it may well be that its relatively higher concentration is responsible for the phototropic and geotropic curvatures which are always found to occur at the nodal points as described by Van Overbeek (1945), Shafer (1939).

TABLE 26

Auxin content of nodes and internodes of Petkus winter rye. 6 weeks vernalised seeds were grown under 17 hours constant day length. Water extraction. Dilution 100 cc.

	Score	Fresh wt. gm.	R.T.F. %	Total Auxin Iaa Eq. µgm per	Auxin conc. IAA Eq. µgm per gm wt.
Young node	37	0.08	75.3	0.63	7.9
"	"	"	74.1	0.69	8.6
Young internode	37	0.08	83.5	0.27	3.4
"	"	"	82.8	0.28	3.5.
Mature node	47	0.08	91.7	0.12	1.5
"	"	"	88.4	0.16	2.0
Mature internode	47	0.08	88.3	0.16	2.0
"	"	"	90.0	0.14	1.8

D I S C U S S I O N

It has been known for a long time that auxin inhibits root elongation. The growth inhibition of intact roots of seedlings has been demonstrated by many workers, for example, Thimann (1936), Lane (1936), Marmer (1937), Bonner and Koefli (1939), Thomsom (1946), Audus (1949 b), Moewus (1952) and Åberg (1950, 1951, 1952) and others. Some of the earlier workers of 1936 reported that a very low concentration of indoleacetic acid slightly accelerated the growth of intact roots. But Marmer (1937), Lane (1936), Bonner and Koefli (1939) failed to get any consistent stimulation of root elongation in IAA. Recently Åberg (1950, 1952) in repeated experiments, with Linum obtained no consistent stimulation of root elongation in concentrations of IAA ranging from 10^{-3} to 10^{-12} M; but Moewus (1952) reported a slight acceleration in Lepidium roots occurring in the range of 10^{-9} to 10^{-11} M of IAA. In this present investigation there was no consistent acceleration of root growth in rye seedlings in either IAA between 10^{-6} to 10^{-13} M or in any plant extract studied.

The mechanism of the inhibiting action of auxin on roots is by no means clear. It has been suggested that the normal auxin concentration in intact roots is slightly supra-optimal (Thimann 1948, Åberg 1952), therefore, the application of more auxin from outside causes growth inhibition. Åberg (1953) has put forward a hypothesis that (1) the auxin (x) exerts its action when loosely combined with some specific portion of the cytoplasm (P). (2) The amount of the active complex (Px) in the normal root is supra optimal for longitudinal growth, and further applications of auxin causes inhibition of increasing intensity.

The inhibitions of the longitudinal growth as he suggests is probably not caused by any slowing down of metabolic processes but rather by a direct influence upon the growth processes. Burstrom (1951) studied the mode of action of auxin on roots. He has specified four different actions that externally added auxin and related compounds may exert on root growth (a) a phase of positive action on the first part of the cell elongation process; (b) an inhibiting action on the second part of the same phase, which comprises the main part of elongation;

(c) an antiauxin action exerted by certain compounds; and (d) an unspecific toxic action exerted by both auxins and anti-auxins involving a reduction of the rates of both cell multiplication and cell elongation. The first two actions do not effect cell multiplication and are antagonised by antiauxins.

The quantitative relationship of auxin with the root growth of seedlings has been adopted as a assay method in this work. From table (3, 4) and Fig. (2) it will be seen that the relation of root inhibition is approximately proportional to the logarithm of concentration of IAA. The measurable inhibition of root growth can be obtained at a concentration as low as 0.001 mg litre of IAA.

In the course of the present work ~~amount~~ auxin has been extracted from the plant tissues either by water or ether at 0°C and has been expressed in terms of IAA equivalent. Although Terpstra (1953) has shown by chromatographic methods that the extract from coleoptile tips probably only contains IAA, and Van Overbeek (1945) has identified the auxin extracted from grass stems at 0°C with IAA; yet it should be born in mind that data in the tables which follow could be affected by "other substances", e.g., growth inhibitors which might be present in the extract and might have an effect on root growth.

The nature of action of native growth inhibitors or antiauxins of plant on root growth is obscure. Åberg (1950, 1951) has studied the action of various synthetic antiauxins on root elongation of flax seedlings. He showed clearly that root elongation was stimulated regularly by antiauxins at low concentrations. He obtained about 20% increase of root elongation over control by eight different α(naphthylmethyl-sulphide) or selenide -alkyl-carboxylic acids at 10^{-6} M concentration; while no such consistent increase of root elongation was found at any concentration of IAA. According to him the consistent increase of root elongation by antiauxins at low concentrations is due to an antagonistic action upon the native auxin of the roots. When antiauxins are applied together with auxin, a marked restorative effect upon the root elongation was noted which suggests that antiauxins are antagonistic towards the action of auxin on root growth.

Auxin content of the excised embryo.

The auxin content of the embryo of rye has been studied in some detail; the results obtained in foregoing experiments and summarised in tables (6-11) and figures (4-8) show that in the absence of the endosperm the embryo can produce its own auxin, and the concentration of auxin (auxin per gm. fresh weight) of the excised embryo is equal to that of the embryo attached to the endosperm. The excised embryo, however, grows more slowly, and in consequence the total amount of auxin produced is less than that of in the unexcised embryo. The slower growth of the excised embryo ~~may~~ might be due to the following causes: (a) mechanical damage during excision, (b) lack of growth factors other than auxin supplied by the aleurone layer and the endosperm of the grain; and (c) limited auxin supply.

(a) Mechanical damage is likely to be small if sufficient care is taken; although there is no reason to suppose that considerable damage occurs during excision, it is impossible to prove that damage due to excision has had no effect. During the early stages of germination (2 day old embryos) there is no difference in growth between excised and unexcised embryos so that if there is any damage its effect appears only in later stages of germination (4-8 day-old embryos).

(b) Under this heading may be considered (1) nutritive factors, including accessory growth factors, (2) water supply. Schander (1934) has shown that the interruption of the connection between the aleurone layer and the embryo reduces the growth of the embryo, and unpublished work of Ropp shows that the separation of the aleurone layer from endosperm leads to reduction of the embryo growth. Purvis (1944) has shown that addition of the ground endosperm to the medium of excised embryo does not influence the growth of the excised embryo. The nutrient supply of the medium on which excised embryos are grown is limited to sucrose at optimal concentration and inorganic nitrogen with other nutrient salts (Table 1), and it may well be that certain accessory nutritive factors are required for maximal growth.

So far as water supply is concerned, the excised embryo is more favourably situated than the attached embryo, as the latter has to withdraw water against the force of imbibation of the endosperm. In the early stage of germination, however, there is no marked difference in growth between excised and attached embryos; it is only at a later stage (after 3 to 4 days) that the marked superiority in growth of the attached embryo is shown. Differences in growth are shown by coleoptiles and roots; but while the overall length of the plumules are about the same, in the excised embryo the internode (which ~~occurs~~ extends in 6-8 days old rye embryos in the dark at room temperature) is longer and the primary leaf is shorter than in attached embryos. [illegible] Evidence appears to favour the view that accessory growth factors transported from endosperm to the embryo are required for optimal growth of the embryo in the later stages of germination.

(c) Auxin is known to be present in endosperm in large quantities as shown by Avery et al (1940, 1942 b) in wheat, oat, maize; Hatcher (1945) in rye, maize; ~~Sircar and~~ Das (1949 ~~1951~~) in rice, wheat, oat, maize. Evidence for movement of a precursor of auxin from base to the tip of coleoptile has been presented by Skoog (1937); and a gradual disappearance of auxin from the grain during germination accompanying the growth of the embryo has been

shown by Sircar and Das (1951). Hatcher (1945) has shown that during the formation of the grain in rye the amount of auxin of the grain extractable by water and alkali hydrolysis rapidly increases and reaches a maximum some 7½ weeks after anthesis, the high level of auxin is however not maintained and during the final ripening both fractions are reduced; in consequence the total auxin is much smaller in the mature rye grain. The ultimate fate of the auxin in the grain is unknown; it is however possible that the unaccountable auxin has been converted into a precursor which does not yield free auxin by hydrolysis. Whether a true precursor is present in the endosperm and is transported to the embryo or not, this cannot be the main source of auxin of the embryo, as total auxin of the excised embryo in the first four days is scarcely less than that of the attached embryo, and moreover the concentration of auxin (auxin per gm fresh weight) remains approximately the same in both excised and unexcised embryos throughout all stages of germination. Whatever may be the missing factor in the excised embryo leading to its reduced growth it does not prevent the synthesis of auxin in the embryo. On the other hand it has been shown in this paper that the production of auxin in the excised embryo is directly related to the concentration of sucrose in the medium

which suggests that sucrose either acts as a substrate for synthesis of auxin or is necessary to provide energy for auxin synthesis. Low temperature (2°c) entirely prevents any auxin production in the excised embryo. The temperature coefficient of auxin production between 2° to 10° c (Q_{10} = 3 approx.) is higher than that between 10° to 20° c (Q_{10} = 1.5 approx.). The optimal amount of synthesis of auxin takes place between 20° c to 25° c. A temperature of 35° c leads to the disappearance of auxin from the embryo. When 2-day or 4-day old embryos were transferred from room temperature (20° c) to high temperature (35° c) their auxin content was found to disappear; but transfer experiment shows that exposure to 35° c for a time does not prevent the resumption of auxin synthesis if the temperature is lowered again. The cause of the disappearance of auxin at high temperature is not very clear. Three possible explanations are suggested (a) auxin already present in the embryo is used up by growth processes and is not replaced, as its production is inhibited at 35° c. (b) It is destroyed by enzymes operating optimally at high temperature. (c) Its activity ~~is inhibited~~ by growth inhibiting substances. There is no evidence, however, that such substances are developed at high temperature, for the temperature relations of their production are as yet unknown.

Furthermore, evidence of growth during and after temperature treatment is not consistent with this assumption.

It has been shown that oxygen is required for synthesis of auxin; therefore, it is probable that aerobic respiration is essential. The effect of respiratory poisons on auxin synthesis has not yet been investigated, therefore, no conclusion can be reached on this point. Auxin appears to be stable under anaerobic condition; auxin of 4-day old embryos did not disappear when they were transferred from air to anaerobic condition. Under anaerobic condition, however, no further auxin was produced nor did growth take place. One important observation should be made in this connection that although embryos have accumulated appreciable amount of auxin during the first 4 days growth in air, no further growth is possible without oxygen. ~~indicating that the effect of auxin on growth depends at some stage on an oxidative reaction.~~ Bonner (1933) found that coleoptile segments immersed in a solution of growth-promo-ting substance elongated only slightly in the absence of oxygen. Van Ameizden (1917) showed that neither geotropic nor phototropic stimuli were perceived under anaerobic conditions. It would therefore appear that the auxin system is rendered inoperative in the absence of oxygen. Thus in anaerobic conditions

no matter how much auxin is present, it is without effect on growth.

Clearly more work will be needed in analysing the factors concerned for synthesis of auxin in the plant, but this investigtion was carried far enough to prove that the excised embryo is an ideal tissue for study of auxin synthesis.

The relation of auxin to growth of the embryo

The relation of auxin to growth of the embryo and of its different parts (e.g., coleoptile, leaf, root, coleoptile node) has been studied. Carbohydrate supply, oxygen and optimal temperature have been proved to be necessary for the production of auxin in the embryo; levels of these factors optimal for growth also proved to be optimal for production of auxin. The total auxin content of the embryo increases with age. During the first four days both the auxin content of embryos and their growth rate increases with rising temperature or sugar or oxygen supply in a similar way; the highest rate of increase of auxin and growth during the first eight days is between the 2nd and 4th day.

Skoog (1937) first estimated the diffusible amount of auxin in the leaf of Avena by the "deseeded" Avena test and he found that the auxin obtained by diffusion into agar blocks from the 4-day old Avena leaf was about 5 percent of the amount obtainable from the coleoptile tip. In the present study the auxin extracted from the 4-day old rye leaf was about 12 percent of the amount obtainable from the whole embryo, and though these two findings are not strictly comparable, yet both show that auxin is present in the leaf as well as coleoptile. Thimann (1934) estimated auxin in roots of Avena seedlings and he detected much higher concentration of auxin in root-tips than in upper segments. Recently Pilet (1951) has studied the native auxin content of roots of lentil at different developmental stages, determined by a carefully calibrated Avena test. This shows that the auxin content of roots increases with increasing length. In this present study the total quantity of auxin in roots and leaves has been shown to increase rapidly, plotted against time the values form smooth curves which parallel the curves for growth (Figure 5, 6). On the other hand the concentration of auxin found in roots and leaves remained almost constant during the period of eight days, while the concentration of auxin in the coleoptile gradually decreased during the termination of its growth.

The whole picture of the auxin relations of the growing embryo suggests that auxin is closely associated with growth. The maximal rate of production of auxin has been found to occur in embryos during the period of maximal growth rate; the rate of auxin production follows a course parallel to the growth rate according as the latter is changed by temperature or by sucrose concentration.

The question arises whether growth is determined by auxin concentration or whether the auxin concentration is a result of growth. It is true that there is a general relationship between the production of total auxin and growth rate when different treatments are compared but concentration of auxin (auxin per gm. fresh weight) is almost independent of treatment. The sigmoid form of the growth curve of the embryo shows that the maximal growth rate occurs between 3 to 4 days from germination while the concentration of auxin in the tissue remains almost the same. Data in table (7) show that mean concentation of auxin of 2-day old embryo at 2% sucrose is 1.11 μgm., while at that of 4-day old embryo is 1.14 μgm., and that of 8-day old embryo is 0.69 μgm.

The increase of growth rate of the embryo up to 4 days, therefore, takes place without any change of auxin concentration; whereas later a fall in concentration of auxin accompanies a steady growth rate. Considering the case of the coleoptile alone, the auxin concentration from 3rd day onwards steadily falls until growth ceases (Figure 7) while in other organs (e.g., leaf and root) the concentration remains more or less constant. The estimation of auxin in the coleoptile has not been made earlier than 3 days from germination, so that it is not known whether the concentration of auxin is higher from the beginning; on the other hand as has already been stated, no change of auxin concentration is found in the embryo between the 2nd and 4th day and if over this period of germination the concentration of auxin of other parts of the embryo also remains constant as it does later, it may be, therefore, inferred that the concentration of auxin of the coleoptile must be high as soon as growth begins; if this should be the case the picture presented by the auxin concentration obtained by extraction is very different from that of the diffusible auxin obtainable from the coleoptile tip. As Van Overbeek (1938) has shown that diffusible auxin of the coleoptile tip of Zea rises and falls during the grand period of growth in a way resembling that of growth rate.

If in rye the diffusible auxin is similar to that of zea as studied by Van Overbeek it must ~~have~~ been inferred that in the early stages of growth auxin must accumulate in the tissue of coleoptile and not be transported from coleoptile tip to agar block.
It may be possible that auxin can only accumulate in the cell to a maximum level and above this level is transported away. This possibility would go far to explain the fact that they growth rate of the embryo is independent of the concentration of auxin while closely related to the total amount extractable.

The relation of auxin to vernalisation.

The locus of vernalisation at low temperature and of devernalisation at high temperature (Gregory and Purvis 1938, Friend Ph.D., thesis L.U. 1953) seems to be in the tissue of the embryo itself. From the study of the auxin content of the excised embryo at vernalisation temperature (2 °C) it has been shown that the total quantity as well as concentration of auxin remain almost unchanged at that temperature. This result is not in agreement with Cholodny's hypothesis (1936, 1939) which suggests the vernalised condition in the embryo is induced by prolonged contact with the auxin-rich endosperm, resulting in a high concentration of auxin in the embryo. The data presented in table (12) show that embryos which are known to be vernalised may contain a very low level of auxin.

Bonner and Thurlow (1949), also Bonner (1949) have observed that flower formation in Xanthium can be suppressed by treating the plant with auxin or synthetic growth substances and can be induced by application with auxin antagonists or agents which destroy the auxin.

Leopold and Thimann (1949) injected auxin at various concentrations through leaves of young Barley plants and concluded that low concentrations of auxin may increase flowering while higher concentrations inhibit. Therefore, it may be suggested that a condition for maintaining a low auxin content in the embryo (so far as temperature is concerned) may accelerate flower initiation. But facts derived from other experiments do not agree with this assumption, as for example, at 10°C auxin content of the embryo does not maintain a constant low level, instead it increases with the growth of the embryo but the vernalisation response still occurs to a significant degree(Purvis 1948). Again the constant low auxin content in the embryo could be maintained in absence of oxygen or of sugar but no response to vernalisation could be obtained in such conditions (Purvis and Gregory 1938, 1944).

Auxin content of the developing plant.

Winter rye is a facultative long day cereal, susceptible to be vernalised during germination or when growing as a young green plant. In this study auxin has been estimated at different stages of both vernalised and unvernalised plants growing under continuous light, long days and short days. The process of the vegetative growth in rye and other cereals falls into two phases: (a) during tillering a continuous production of leaves and laterals (b) and after floral initiation a rapid extension of the stem. It is well known that tillering falls off at the time of differentiation of the ear. Maximum extension growth of the stem is also completed before anthesis.

In the foregoing experiments a close relation of the auxin content of the plant to the extension growth of the stem and development of the ear has been revealed. Irrespective of treatments little difference in auxin content has been noted in the growing stem or undifferentiated apices or developing ear when compared at the same stage of floral development ("scores"); thus the auxin balance of a plant is independent of its age on a time scale;

and depends only on the particular stage of development reached. This suggests that the level of auxin in the plant is also controlled by the environmental factors e.g., photoperiod and temperature in the same way as growth and development.

Auxin content of apices before and after floral initiation

The total quantity of auxin in undifferentiated apices (each weighing approx. 0.05 mg. fresh weight) is naturally extremely small but its concentration (auxin per gm. wt.) is very high. The auxin content of apices of plants growing in short days is higher than that of plant apices under long days or continuous light. The slight increase in auxin content of apices which occurs at the beginning of initiation (score 21-23) is not consistent; later the total auxin in apices gradually increases when they form stamen initials and undergo further development. The extremely high concentration of auxin in undifferentiated apices and the lack of evidence for its rapid decrease during initiation is contrary to the assumption of Leopold and Thimann (1949) that auxin increases flowering at a low concentration.

Auxin content of the stem.

After floral initiation of the plant a large amount of auxin appears in the young stem (score 34-35) in which rapid extension is just beginning.

Before reaching the score 34-35 neither the crown of the plant nor the first extending internode of the stem of scores 28-30 (which is rather difficult to differentiate from the crown) contains as much auxin as does the stem of plants "scoring" 34-35 approaching the grand period of growth. (Table 20).

The accumulation of auxin in the stem continues until the flag leaf is out. After ear emergence total auxin does not increase in the stem. On the other hand its concentration decreases rapidly until anthesis; this decline of auxin concentration in stem shows an approximately linear relationship with the extension growth of the stem (Figure 19).

Wittwer (1943) found that an appreciable amount of auxin accumulated in the carpel of Zea after anthesis and he emphasised the phenomenon of reduction division as a cause of such accumulation.

TABLE 27

Auxin content of the basal crown and young stem of Petkus winter rye. Ether extraction. Dilution 100 cc.

Sample	Fresh wt. gm.	Root length of test plant as % of control	Total Auxin IAA Eq. µgm. per stem or crown	Auxin conc. IAA Eq. µgm. per gm. wt.
Crown of the plant before floral initiation. Score Below 21.	0.38	75.7	0.059	1.55
Young stem with a portion of crown at the base, score 28-30.	0.18 0.15	82.2 84.7	0.030 0.024	1.70 1.60
Crown of the plant at the end of stem extension. Score Past 49.	0.45 0.56	99.0 97.8	0 0	0 0

In this study it has been shown that the main bulk of the auxin appears in the stem after floral initiation, concentration of which gradually decreases with extension growth. Apparently this phenomenon of appearance of auxin in the stem bears no relationship with the reduction division of the carpel, on the other hand it shows a relation to the onset of the grand period of growth of the stem.

Avery (1937) found in Aesculus and Malus that about a fortnight before starting of spring growth a large amount of auxin accumulated behind the dormant bud and when the bud expanded and the stem started to extend the concentration of auxin in the stem decreased rapidly. In the present work the decline of auxin concentration with extension growth has been demonstrated more clearly in the study of internodes. Internodes of cereals extend in acropetal succession. The auxin concentration is higher in the young unextended internodes near the ear than in the lower internodes which have extended; the concentration gradually decreases with extension growth (Figure 21). The total quantity of auxin after reaching a peak value also decreases; as some of the basal internodes show little or no auxin at the time of anthesis when they are fully extended.

The nodes contain much more auxin than do the internodes, but the amount decreases as the nodes attain maturity. These facts may lend support to the assumption that the seat of the production of auxin of the cereal stem is the meristem of the node. The main bulk of the auxin may come from nodes which in turn directly accelerate the extension growth of the neighbouring internodes. The limitation of the meristematic activity of the node with the age also terminates the production of auxin and stops extensions.

Auxin content of the ear

In the developing ear itself a fall in auxin concentration has been noted during the grand period of elongation (scores 31 to 37). After ear emergence a rise in concentration of auxin in the ear parallels the maturing of another and carpel up to the stage of anthesis. The total quantity of the auxin in the ear, on the other hand, has been found to increase rapidly and significantly throughout the period of the development of the ear.

The results so far discussed here suggest that auxin is more closely associated with extension growth of the stem and development of the ear than with the process of flower initiation as there is no evidence of a significant change in the auxin balance of plant immediately before and after floral initiation. The auxin of the vernalised and unvernalised plants does not differ much when they are compared at the same stage of development, and it has been shown that the auxin level depends entirely on the stage reached (scores) independently of the time taken to reach that stage. In short day conditions when the floral development is retarded and extension growth is inhibited the accumulation of auxin also inhibited. (Table 21, 22).

Transfer experiment.

An important result is deduced from a series of transfer experiment that though short day condition inhibits stem extension of rye (Gott. 1950. Ph.D. Thesis L.U.) yet if the plant can attain a certain stage of development and accumulate a certain level of auxin in the stem the further extension growth of the stem is possible in short days, though the degree of extension will be reduced to a significant level. Thus the high accumulation of auxin associated at the score 33-34, which in rye preceeds the grand period of ear and stem growth, marks a critical stage in development, beyond which the ability to complete ear formation and extension growth of stem is established.

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