EFFECT OF AUXIN ON CHLORELLA VULGARIS

AND

STUDIES ON THE MOVEMENT OF LEAVES

THESIS BY

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GENERAL SUMMARY

Part 1

Chlorella vulgaris of a re-isolated clone was cultured in Knop's solution to which different amounts of pure heteroauxin were added. The results show that auxin promotes the enlargement of the individual cells. Particularly in the young cultures the cell volume is proportional to the logarithm of the auxin concentration. In older cultures the matter is complicated by the shortage of another factor, presumably food. At high concentrations auxin retards cell division. This is probably due to a decreased amount of chlorophyll and a decreased photosynthetic activity.

In agar medium heteroauxin in very low concentrations stimulates growth, but at high concentrations inhibits growth of the alga.

Auxin does not affect respiration but it markedly affects the rate of photosynthesis. The effect is indirect through the changes in the chlorophyll content and the cell size. In the young cultures the rate of photosynthesis is proportional to the chlorophyll concentration, while in the old cultures it is proportional to both the chlorophyll concentration and the extent of the cell surface.

Part 2

Malva leaves are diaphototropically sensitive, i.e. the leaves orient themselves transversely to the sun's rays. They follow the sun's course during the day and return to their original position at night. No such movement can be observed in diffuse light or in darkness. Light is perceived by the

lamina. The movement consists of a curvature of the laminar joint which is capable of reversible contraction and extension. A difference in osmotic pressures on the two sides of the petiole is found to be responsible for the curvature.

The leaves of Carica papaya assume a horizontal position in the day and droop abaxially at night. The movement has a normal periodicity of twenty four hours due to some internal cause (autonomic).

The mechanism of the movement has been shown to be of the following sequence:

(1) There is a differential distribution (or production) of auxin on the apical and basal lobes of the leaf blade.

(2) Because of its special vesicular structure the auxin from the apical lobe goes to the lower (abaxial) side of the petiole, while that from the basal lobe goes to the upper (adaxial) side of the petiole.

(3) Because of the differential supply of auxin, the two sides of the petiole grow unequally and give rise to nyctinastic curvature.

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TABLE OF CONTENTS

PART I

Effect of Auxin on Chlorella vulgaris

Chapter I. Effect of auxin on the growth of Chlorella vulgaris in liquid medium.

I. Introduction.

II. Material and method. 1. Isolation of material 2. Method of culture

III. Determination of volume concentrations.

IV. Determination of dry weight.

V. Determination of cell size.

VI. Determination of cell number.

VII. Determination of auxin content.

VIII. General discussion.

IX. Summary.

Chapter II. Effect of auxin on the pigment content, respiration and photosynthesis of Chlorella vulgaris.

I. Introduction.

II. Determination of chlorophyll.

III. Determination of carotenoids.

IV. Respiration.

V. Photosynthesis.

VI. Relation between photosynthesis, chlorophyll content and the surface of the cell.

VII. Summary.

Chapter III. Effect of auxin and auxin-like compounds on the growth of Chlorella vulgaris in solid medium.

I. Introduction.

II. Material and method.

III. Results.

IV. Conclusion and discussion.

1. Heteroauxin.

2. Auxin-like acids.

3. Vitamine B_1.

V. Summary.

TABLE OF CONTENTS

PART II

Studies on the Movements of Leaves

Chapter IV. Diaphototropic movement of leaves of Malva neglecta.

I. Introduction.

II. Effect of light on the movement.

A. Observations under normal light conditions.

B. Observations under evenly diffused light.

C. Observations in darkness.

D. Observations under colored light.

III. Analysis of the movement.

A. The organ of perception.

B. The organ of reaction.

IV. Mechanism of the movement.

A. Relation to growth.

B. Extension and contraction of the laminar joint.

C. Osmotic relations in the joint.

V. Summary of results and discussion.

VI. General summary.

Chapter V. Nyctinastic movement of leaves of Carica papaya -- nature of the movement.

I. Introduction.

II. Material and method.

III. The normal ~~cause~~ course of the movement.

IV. Movement under constant illumination.

A. Leaves grown in the green house.

B. Leaves grown in constant light.

I

Effect of Auxin on the Growth of Chlorella vulgaris in Liquid Medium.

I. Introduction.

Within the last ten years the literature on auxin, the plant growth hormone, has piled up with amazing rapidity. The general results have been recently summarized in a number of reviews (Went 1935, Thimann 1935) and a book bearing that title (Boysen-Jensen 1936). Surveying this field one will find that by far the greater part of the work on auxin has been done with higher plants. Very little is yet known as to its relation in lower plants. A few papers have reported the occurrence of auxin in fungi (Nielsen 1930, 1932) and algae (Van der Weij 1933), but its effect in these groups of plants is almost unknown. In fungi it appears to be ineffective (Boysen-Jensen 1932). In some algae (Euglena) it seems to stimulate cell-division and germination of cysts (Popoff 1935). In a recent abstract (1936) Elliot reported some experiments with auxin on the growth of protozoa. He found that auxin promotes the growth of chlorophyllous protozoa (protophyta) but is without effect in the colorless species. A few papers have also been put out by Leonian and others (1935, 1936, 1937) but their data are too crude to be of any value. Furthermore the authors appear to have a rather insufficient knowledge of the question and have drawn quite erroneous ~~and ridiculous~~ conclusions. It seems to be important therefore to make a careful study of the subject. This is the purpose of the present study.

The unicellular green alga Chlorella vulgaris has been chosen as material because the physiology of this form is well known. The growth cycle of the species has been recently studied by Pearsall and Loose (1937). The metabo-

lism (Beckwith 1929), respiration (French, Kohn, and Tang 1934), photosynthesis (Emerson 1936) and nitrate reduction (Warburg 1928) of this and other species (C. pyrenoidosa) of Chlorella have been investigated. Because of this it is a suitable object for the study of the effect of auxin and the mechanism of the processes of growth.

II. Material and methods.

A. Isolation of material.

A pure strain of Chlorella vulgaris that had been cultured in the laboratory for more than ten years was kindly supplied by R. Emerson. Preliminary examination of the culture showed that the size of the individual cells varied considerably. The frequency distribution curves of the measured diameters were not symmetrical and had two to three indistinct peaks. See figure 1.

Table I. Distribution of cell size in the original culture.

Size class	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15
Frequency	0	14	20	35	79	109	97	105	82	84	65	21	7	1	1

This was probably due to the occurrence of mutations which arose during long cultivation. In order to get a more uniform strain the culture was diluted 10^2, 10^3, 10^4 and 10^5 times and spread on nutrient agar plates. In a month small colonies appeared which were probably derived from the division of single cells. The colonies were selected and transferred to agar slants in test tubes, and allowed to grow for about two weeks. A total of fifty-four slants was thus obtained from which nine were selected and transferred to liquid media. One of these cultures (A-15a) was chosen as the inoculum for all the experiments in the present investigation. The cell size in this clone (A-15a) fell on a normal distribution curve as shown in table II and figure 2.

Table II. Distribution of cell size in clone A-15a.

Size class	0-1	1-2	2-3	3-4	4-5	5-6	6-7	Total --- 988 cells.
Frequency	4	204	513	221	32	11	3	Average diameter -- 3.12 units.

follows p. 2

Table II. Cell size distribution in clone A-15a.

Units in micrometer divisions.

Ocular 10x

Objective 40x

Size class ----	0-1	1-2	2-3	3-4	4-5	5-6	6-7	Total	Average diam.
Frequency ----									
1st measurement ---	1	119	342	137	15	7	3	624	3.13
2nd measurement ---	3	85	171	84	17	4	0	364	3.10
Total ----------	4	204	513	221	32	11	3	988	3.12

B. Method of culture.

The culture medium described by Warburg and Negelein (1922) was used. Four stock solutions were prepared from "Kahlbaum" chemicals in redistilled water from a Pyrex still.

(1)	$MgSO_4 \cdot 7H_2O$	50 gm. per liter
(2)	KNO_3	25 " " "
(3)	KH_2PO_4	25 " " "
(4)	$Fe_2(SO_4)_3 \cdot 7H_2O$	2.8 " " "

The solid medium used in the plates and slants was made according to a modified formula of Benecke which consisted of:

(1)	KH_2PO_4	14 c.c. of stock
(2)	KNO_3	6 " " "
(3)	$MgSO_4$	4 " " "
(4)	$Fe_2(SO_4)_3$	trace
(5)	H_2O (dist.)	1000 c.c.
(6)	agar	2 %

The liquid medium consisted of:

(1)	$MgSO_4$	10 c.c. of stock
(2)	KNO_3	10 " " "
(3)	KH_2PO_4	10 " " "
(4)	$Fe_2(SO_4)_3$	0.2 c.c. of stock

Distilled water was added in ammounts to make up the volumes shown in column 2 of table III for the different cultures. The medium was placed in 300 c.c. Erlenmeyer flasks with entrance and exit tubes for gas as described by Emerson (1929) and sterilized in an autoclave for fifteen minutes under eighteen pounds of pressure. A stock solution of 0.2 mg. per c.c. pure crystalline heteroauxin

(beta-indole-acetic acid Merck) was made up fresh for each experiment. This auxin stock was sterilized separately, and then added to the culture flasks in amounts given in column 3 of Table III. This brought the total volume of each culture to 200 c.c. of approximately equal osmotic pressure (concentration).

Table III. Auxin concentrations of different cultures.

Culture No.	Media c.c.	Auxin Sol. c.c.	Resulting auxin conc. mg./c.c.
1	200	0	0
2	199	1	0.001
3	195	5	0.005
4	190	10	0.010
5	180	20	0.020
6	160	40	0.040

The media were then inoculated with one c.c. (3-4 $mm.^3$ of cells) of the original stock cultures (A-15a) by means of sterile pipettes. The cultures were grown in a glass-bottom water bath and illuminated from below with a series of internally frosted filament lamps. A continuous stream of air containing 5 % CO_2 was passed through the cultures. The culture conditions of the different experiments are given below.

Table IV. Experimental conditions.

Exper. No.	Date	Days of culture.	Culture No.	Lighting conds.	Temperature of tank.	Remarks on growth.
1	8/14-10/28	75	14	9x40 watts	warm	very slow
2	11/12-11/27	16	9	"	medium	fair
3	12/20-1/12	25	7	"	cold	slow
4	2/1 -2/16	15	7	9x60 watts	cold	fair

Exper. No.	Date	Days of culture.	Culture No.	Lighting conds.	Temperature of tank.	Remarks on growth.
5	2/24-3/11	16	7	9x60 watts	cold	good
6	3/18-3/25	7	6	"	cold	good
8	3/29-4/13	15	6	"	cold	good

It can be seen from the table that the external conditions were not at all the same in the different experiments. The first three experiments had a lower light intensity and a much more varied environment than the last three experiments. Therefore the different cultures of each experiment were strictly comparable only within each other and only a rough comparison was allowable with the last three experiments where the conditions were more uniform.

At the end of the experiment the cultures were harvested and determinations were made of the volume concentration, dry weight, average cell diameter, cell number, and the auxin concentration. For convenience these determinations will be discussed separately in the following sections.

III. Determination of volume concentration.

The volume concentration, expressed in $mm.^3$ of cells per c.c. of culture, was determined by centrifuging the cells from a known volume of the culture in a graduated hematocrit. The centrifuge was run at full speed (greater than 3000 r.p.m.) for twelve minutes, a time previously found to give a constant reading. At least two determinations were made for each culture. An example of such a determination is shown in table V. A summary of the results of different experiments is found in table VI.

It will be seen that the deviation for duplicate readings (table V) was about 2 - 3 % although it might be higher for more dilute cultures. The deviation between two duplicate cultures was higher (5 %) especially

Table V. Volume determination by centrifuging. (Exper. 5, March 11, 1937).

Culture No.	Auxin conc.	Hematocrit No.	Culture taken c.c.	$mm.^3$ cell read	$mm.^3$ cell correct.	$mm.^3$ cell /c.c. cul.	Average
1a	0	1	6	15.7	27.8	4.63	
	0	8	6	28.0	27.3	4.55	4.59
1b	0	1	6	14.8	25.8	4.30	
	0	8	6	27.0	26.2	4.37	4.33
2	0.001	13	6	26.0	24.7	4.12	
	0.001	14	6	25.5	24.6	4.10	4.11
3	0.005	1	6	14.7	25.6	4.27	
	0.005	13	6	27.0	25.8	4.30	4.28
4	0.010	8	6	24.9	23.8	3.97	
	0.010	14	6	24.0	23.0	3.83	3.90
5	0.020	13	7	18.5	16.9	2.41	
	0.020	14	7	18.0	16.8	2.40	2.41
6	0.040	14	100	26.0	24.8	0.25	0.25

Table VI. Volume determinations by centrifuging -- summary of experiments.

Exper. --		6	4	8	5	2	3	1
Days ----		7	15	15	16	16	25	75
Culture No.	Auxin conc.	$mm.^3$ cells per c.c. culture						
1a	0	1.22	3.55	4.41	4.59	1.09	3.13	3.71
1b	0	1.42	-	-	4.33	-	2.93	3.50
2	0.001	1.51	3.80	4.27	4.11	0.87	5.05	4.32
3	0.005	0.25	2.24	3.40	4.28	0.64	4.44	3.65
4	0.010	0.16	2.67	3.41	3.90	0.54	4.88	3.70
5	0.020	0.16	-	0.10	2.41	0.11	4.74	3.98
6	0.040	-	0.27	-	0.25	0.16	0.15	3.90

for the young rapidly-growing cultures, which was probably due to the slightly different ammount of inoculation.

A general conclusion can be drawn from these results. This is that in low concentrations of auxin (0.001 mg./c.c.), the hormone does not affect the growth of the cultures but in higher concentrations (0.010 to 0.020 mg./c.c.) it progressively retards the growth of the cultures, so that at the highest concentration (0.040 mg./c.c.) the culture fails to grow at all. Experiments 1 and 3 were exceptional (section VIII, table XVI) in that auxin had disappeared by the end of the experiment. Experiment 2 was also abnormal in this respect; the cultures were dilute and the destruction of auxin was high. These three experiments had different culture conditions (table IV) and also a different source of heteroauxin (prepared by the chemical laboratory of the institute). Culture 6 presented a pathological condition, due to an overdose of auxin. The cells were brownish red and disintegrating.

It is known that the growth of Chlorella in culture slackens after the concentration reaches a stage of saturation. This phenomenon is not due to the exhaustion of the mineral supply of the medium (Pearsall and Loose 1937) (Sargent, unpublished result) but is perhaps a result of decrease in photosynthetic activity, the only means by which the plant can obtain its food in the inorganic medium. At this stage there is a decrease in hexose content and protein synthesis with an accumulation of wall material, comparable to the phenomenon of senescence of the cells of the higher plants (Pearsall and Loose 1937). On account of this lower rate of growth, the high concentration auxin cultures reached saturation considerably later than the controls (table VI). In this respect auxin may

be regarded as ~~able to keep~~ keeping the cells longer in the juvenile state ~~as it is able to prevent aging in the higher plants (Went 1935).~~ A fuller discussion of this will be given later.

IV. Determination of dry weight.

Dry weight determinations were made on 100 to 200 $mm.^3$ of cells from each culture. The cells were centrifuged out of the culture medium, washed in distilled water, transferred to a weighing bottle and dried in an electric oven at 60 - 70°C. Table VII gives an example of such a determination and tables VIII and IX give the summary of the results of three experiments in which the dry weight is expressed respectively as mg. per c.c. culture or mg. per $mm.^3$ cells.

Table VII. Dry weight of cells. (Exper. 5, March 11, 1937)

Culture No.	Auxin conc.	Weighing bot. No.	Culture taken c.c.	Cells $mm.^3$	Tare wt. of bot.	Wt. of bot. plus cells.	Dry weight cells mg./c.c.
1b	0	2	50	216.5	42218.1	42255.1	0.740
2	0.001	31	50	205.5	41397.5	41434.4	0.738
3	0.005	30	50	214.0	40496.2	40534.0	0.756
4	0.010	8	50	195.0	37865.6	37898.7	0.662
5	0.020	23	70	168.7	39973.4	40004.4	0.443

Table IX. Dry weight per $mm.^3$ cells.

Exper. --		8	5	1
Days ----		15	16	75
Culture No.	Auxin conc.			
1a	0	0.174	0.171	0.194
1b	0	-	-	0.182
2	0.001	0.181	0.180	0.173
3	0.005	0.207	0.177	0.182

Table XIII. Dry weight per c.c. culture.

Exper. --	8	5	1
Days ----	15	16	75
	0.766	0.740	0.720
	-	-	0.639
	0.774	0.738	0.743
	0.705	0.756	0.667

Table IX (Con.)

Exper. --		8	5	1
Days ----		15	16	75
Cult. No.	Auxin conc.			
4	0.010	0.196	0.170	0.171
5	0.020	-	0.183	0.170
6	0.040	-	-	0.172

Table VIII (Con.)

Exper. --		8	5	1
Days ----		15	16	75
Cult. No.	Auxin conc.			
4	0.010	0.669	0.662	0.633
5	0.020	-	0.443	0.678
6	0.040	-	-	0.670

A study of the tables shows that the dry weight, i.e. the amount of dry matter per c.c. of culture decreases with the increasingly high concentration of auxin (table VIII), so that the dry weight per $mm.^3$ of cells is approximately constant within the limits of experimental error (table IX). This result indicates that auxin does not affect the water content of the cells and the increase in cell volume caused by auxin (see next section) is not exclusively due to the absorption of water. This may be due to an immediate increase in wall material after expansion (Söding 1934). Or more probably because in Chlorella, which is autotrophic, an increase in cell volume by water absorbtion is quickly followed by an increase in assimilation as will be shown in a later chapter (Chapter II).

V. Determination of cell size.

A. Method of measurement.

Cell size was determined by measuring the diameters of a large number of cells with the aid of an ocular micrometer under high magnification (880x). A preliminary experiment (table II, figure 2) showed that one sample in which 300 cells were measured was enough to give a good distribution curve and a reliable average. The same procedure was therefore followed in all the later experiments*.

Several precautions were observed in the measurement:

* In experiment 2, 1000 cells were measured.

(1) Measurement was made as soon as possible after the cultures were harvested.

(2) A thin slide preparation was used so that all the cells could be seen at the same focus.

(3) Several spots were taken at random on the slides and all the cells at ~~that~~ each spot were measured.

(4) The rare ellipsoidal cells were measured along whatever diameter in line with the micrometer.

(5) Cell clusters were measured as one single cell if the enclosing wall was present, and as separate cells if the wall no longer existed.

After measurement a frequency table and a distribution curve were constructed for each culture. An example is given in table X and figure ~~5~~ 3. The mean diameter was then calculated from the frequency table. The probable error of the mean was very small, about $\pm 0.05 - 0.10 \mu$ or less than 2 %. The difference between the means obtained from two measurements was about 1 % and the difference between the mean diameters of two duplicate cultures was 1 % for the mature cultures and about 5 % for the young developing cultures. The diameter of the cells was therefore a much better criterion for a culture than the volume concentration or the dry weight measurements for the study of auxin effects.

The surface area and the volume of the cells were calculated from the mean diameter, assuming the cells to be spherical.

B. Results and discussions.

The average diameter, surface and volume of the cells in four experiments are summarized below (table XI).

Table X. Measurement of cell diameters. (Exper. 6, 7-day old culture.)

Culture No.	1a		1b		2		3		4		5		
Auxin conc.	0		0		0.001		0.005		0.010		0.020		
Size class.	f	%	f	%	f	%	f	%	f	%	f	%	
0-1	0	0	0	0	0	0	0	0	0	0	0	0	
1-2	4	1.1	5	1.4	0	0	0	0	0	0	0	0	
2-3	34	9.5	30	8.4	7	2.0	2	0.8	2	0.7	0	0	
3-4	67	18.7	58	16.2	25	7.1	10	3.8	8	2.7	5	1.9	
4-5	103	28.8	101	28.2	62	17.5	22	8.4	22	7.4	17	6.3	
5-6	89	24.8	75	20.9	102	28.8	36	13.8	37	12.4	29	10.8	
6-7	39	10.9	54	15.1	84	23.7	57	21.8	66	22.1	43	16.0	
7-8	17	4.7	22	6.1	48	13.6	47	18.0	57	19.1	48	17.9	
8-9	3	0.8	10	2.8	16	4.5	30	11.5	36	12.1	38	14.2	
9-10	1	0.3	4	1.1	4	1.1	20	7.6	21	7.0	27	10.1	
10-11	1	0.3			2	0.6	11	4.2	16	5.4	17	6.3	
11-12					1	0.3	7	2.7	10	3.4	17	6.3	
12-13					1	0.3	5	1.9	8	2.7	13	4.8	
13-14							6	2.3	5	1.7	6	2.2	
14-15							5	1.9	6	2.0	5	1.9	
15-16							1	0.4	2	0.7	2	0.7	
16-17							0	0	0	0	1	0.4	
17-18							2	0.8	0	0			
18-19									1	0.3			
19-20									1	0.3			
Total --	358		359		352		261		298		268		
Av. diam.	4.77		4.99		5.87		7.54		7.74		8.21	micrometer scale	
	6.34		6.62		7.80		10.05		10.30		10.95	mu	
Volume	133.5		151.9		248.5		533.2		572.2		689.5	mu^3	
Surface	126.3		137.7		191.2		318.1		333.2		377.5	mu^2	

Table XI. Average cell diameter, surface and volume of cells measured by micrometer. Summary of experiments.

Exper.---		6			2			8			5		
Days ----		7			16			15			16		
No.	Auxin conc.	Diam. μ	Surf. μ^2	Vol. μ^3	Diam. μ	Surf. μ^2	Vol. μ^3	Diam. μ	Surf. μ^2	Vol. μ^3	Diam. μ	Surf. μ^2	Vol. μ^3
1a	0	6.34	126.3	133.5	5.34	89.6	79.7	6.44	130.4	140.0	6.55	134.6	146.8
1b	0	6.62	137.7	151.9	-	-	-	-	-	-	6.48	131.9	142.3
2	0.001	7.80	191.2	248.5	7.41	172.5	213.0	6.33	126.0	132.9	7.46	175.0	217.7
3	0.005	10.05	318.1	533.2	7.48	175.7	219.1	7.32	168.2	205.0	7.37	171.3	210.7
4	0.010	10.30	333.2	572.2	7.31	167.8	200.0	7.99	200.5	266.9	8.36	220.1	306.8
5	0.020	10.95	377.5	689.5	8.45	224.4	316.0	-	-	-	9.52	285.0	452.4
6	0.040	-	-	-	6.71	141.4	158.1	-	-	-	8.58	231.4	330.9

In all experiments the cells in the auxin cultures were much larger than the controls. The average volume ~~is~~ was sometimes as great as four times and giant cells 50 - 60 times that of normal cells.

In figure 4 the average cell volume is plotted against the logarithm of the auxin concentration. It is seen that in young cultures (curves A and B) the cell volume is a linear function of the logarithmic auxin concentration. In this respect Chlorella is comparable to the cells of higher plants. Laibach (1935) found that the size of swellings or ~~calli~~ calluses on decapitated Vicia faba epicotyl increases proportionally to the logarithm of the auxin concentration applied.

In the older cultures (curve C) this relation between cell volume and auxin concentration is more complicated. First, the cell volumes are in general smaller than those of young cultures. Second, while in the high auxin concentration cultures (0.01 - 0.02 mg./c.c.) the logarithmic relation is retained, in the low auxin concentration cultures (0.001 - 0.005 mg./c.c.) the cell volume appears to be constant, although still

above the controls.

It has been said in connection with volume concentration (section III) that the high auxin cultures grew more slowly and reached saturation later than the controls. By referring to table VI it is seen that while the controls and the low auxin cultures had reached the saturation point in 15 or 16 days (Exper. 2 was abnormal, see below) the higher auxin cultures were still well below that stage. It has also been suggested that the slackening of growth at saturation was probably due to a shortage of the food factor caused by a lower rate of photosynthesis. Taking these things into consideration, it is at once evident that the growth in cell volume depends not only on auxin concentration but also on the amount of another factor, presumably food. In the low auxin cultures the food factor was limiting, while in the high auxin cultures this stage had not yet been reached. Here again Chlorella growth is comparable to that of higher plants. The importance of the food factor in the latter has been studied by Went (1935) and Schneider (unpublished results).

The results of experiment 2 require some explanation. Referring to table XVII one can see that the destruction of auxin in all the cultures of this experiment was abnormally high (50 - 60 %). The reason for this has been suggested before (section III). Now if one takes the final auxin concentration instead of the initial and shifts the points correspondingly (indicated by arrows on curve C) it can be shown that they agree completely with the results of other experiments. A similar explanation applies to the first point (lowest auxin culture) of experiment 8. In this culture auxin had disappeared completely (table XVII) and the cell volume was therefore equal to the control.

VI. Determination of cell number.

In three experiments cell counts were made with a hemacytometer. Two samples were used for each culture and 500 - 1000 cells were counted in each sample. In experiments 5 and 6 the whole hemacytometer chamber including the grooves was filled, while in experiment 8 only the plateaus were filled with the culture. With the former method the deviation of the duplicate countings amounted to as much as 20 %, while with the latter method the maximum deviation was around 7 %.

Table XII gives an example of such an experiment and table XIII gives the results of three experiments. (See next page).

It is seen that with lower auxin concentration cultures the number of cells per c.c. of culture was comparable to the controls, but in the higher auxin concentration cultures the number was considerably smaller. Therefore auxin in high concentrations, while it promotes the growth of cell volume, retards the growth by division. The latter effect was more pronounced than the former, resulting in a lower volume concentration determined by centrifuging.

Table XIV shows a comparison between the volume concentration calculated from diameter measurements and that determined by centrifuging. In this calculation the cells were assumed to arrange themselves in the manner of closest packing, so instead of the ordinary formula for the volume of a sphere ($4.189\ r^3$), the formula $5.66\ r^3$ was used to allow for the spaces between the packed cells (Bragg and Bragg 1933).

It can be seen that the observed (centrifuged) volume concentrations are much larger than the calculated ones, actually about two times in case of small cells (cultures 1 and 2) and 1.5 times for the larger cells (cultures 3 and 4). As the discrepancy exceeded the maximum error of

follows p. 13

Table XII. Cell counts. (Exper. 8, 15-day culture, April 13, 1937).

Culture No.	Auxin conc.	Cell no./0.1mm.^{3} A	B	C	Average	% dev.	No. cells per cc. cul.	No. cells per mm.^{2} of cells.
1.	0	1155	1155	-	1155	0	11.55 x 10^6	2.61 x 10^6
2.	0.001	1043	1138	1177	1119	7	11.19	2.62
3.	0.005	819	814	-	816	3	8.16	2.40
4.	0.010	614	653	-	634	3	6.34	1.86

Table XIII. Number of cells per c.c. culture. Summary of experiments.

Exper. --		6		5		8	
Days ----		7		16		15	
Cult. No.	Auxin conc.	A	B	A	B	A	B
1a	0	3.61	2.96	6.40	1.40	11.55	2.61
1b	0	3.53	2.49	7.10	1.64	-	-
2.	0.001	3.41	2.26	5.99	1.46	11.19	2.62
3.	0.005	0.24	0.96	6.01	1.40	8.16	2.40
4.	0.010	0.16	1.00	5.18	1.33	6.34	1.86
5.	0.020	0.14	0.88	2.76	1.14	-	-

A -- number of cells per c.c. culture x 10^6
B -- " " " " mm.^{3} of cells x 10^6.

Table XIV. Comparison of cell volume determined by cell measurement and by centrifuging. (Exper. 8, 15-day culture, April, 13, 1937).

Culture No.	Auxin conc. mg./c.c.	Average cell radius mm.$^{-3}$	Cell vol. closest packing mm.$^{-9}$	Calc. vol. conc. mm.^{3}/c.c.	Observed vol. conc. mm.^{3}/c.c.	No. of cells/ c.c. of cult.10^6	Comparison observed / calc.
1.	0	3.22	189.2	2.18 ~~11.55~~	4.41	11.55	2.0
2.	0.001	3.17	179.5	2.01	4.27	11.19	2.1
3.	0.005	3.66	276.9	2.25	3.40	8.16	1.5
4.	0.010	3.99	360.6	2.30	3.41	6.34	1.5

the three determinations by almost ten times, evidently it could not be due to the inaccuracies of the measurements. It is probable that the centrifuged cells had not approached the condition of closest packing. The fact that the two values of volume concentration came closer to each other for larger cells indicated that capillary phenomena perhaps played an important role in the centrifuging.

VII. Determination of the auxin content of the cultures.

In experiments 1 and 2 the Avena test method (Went 1928) was used to determine the auxin concentration in the culture media. In later experiments (experiments 3 to 8) the pea test method (Went 1934) was chosen because of its ease of manipulation and its applicability to a wide range of concentrations. The sterile stock solution of auxin was first tested in several different ~~solutions of various~~ dilutions. The pea curvatures obtained were plotted against the logarithms of the auxin concentration in mg. per 10 liters. (Table XV, Fig. 5)

Table XV. Activity of sterilized stock solutions of G.S. determined by the pea test method.

Dilutions tested.	Concentration mg./10 l.	Log of conc.	Exper. 3	Exper. 4	Exper. 5	Exper. 6	Exper. 8
10	200	2.3	-	497	472	-	570
20	100	2.0	-	-	383	478	-
40	50	1.7	-	-	-	320	-
50	40	1.6	-	394	300	-	-
100	20	1.3	333	298	-	302	345
200	10	1.0	261	-	224	203	-
400	5	0.7	-	-	-	115	-
500	4	0.6	152	-	-	-	155
1000	2	0.3	-	-	82	66	70

The culture media were centrifuged from the cells and tested either as such or in different dilutions. Their auxin concentrations (in mg. per 10 liters) were obtained from their pea curvatures by interpolation from the graph (figure 5). From these results the percentage of auxin which had disappeared was calculated.

Table XVI. Auxin destruction in cultures.

Exper. --		6	4	8	5	3	2	1
Days ----		7	15	15	16	25	15	75
Cult. No.	Auxin conc.	% of auxin disappeared						
2	0.001	32	0	100	0	100	72.6	100
3	0.005	12	0	0	0	100	56.2	100
4	0.010	0	0	0	0	100	53	100
5	0.020	0	0	85	0	94	66	100
6	0.040	-	85	-	95	99.5	56.5	100

Table XVI gives the results of seven experiments. In the first three experiments the destruction of auxin was 50 - 100 % in all cultures. But in later experiments no appreciable destruction was observed except in a few cultures. This was because of the different conditions of the experiments or more probably because of the differences in the sources of the auxin used (section III). In this respect the first three experiments may be regarded as abnormal.

The fact that in normal cultures no detectable amount of auxin disappeared suggests that the amount of auxin actually used up in growth was very small. The disappearance of auxin must be largely due to destruction. The highest auxin cultures (culture 6) in all the experiments showed considerable destruction. The cells in these cultures were

brownish and disintegrating and had probably a high oxidation activity. Some destruction was also found in the lowest auxin culture of experiment 8, due possibly to infection, although no bacterial or fungal growth had ever been noted.

An experiment was conducted to ascertain the destruction of auxin by sterilization. The results are given in table XVII. It is seen that the destruction is about 28 %.

Table XVII. Loss of activity of auxin by sterilization. (Exper. 3, Jan. 12, 1937, stock 0.2 mg./ c.c.).

Dilution	Conc, mg./10 liters C_1	Log C_1	Curvature° unster. x_1	Curvature° ster. x_2	$\frac{x_2}{x_1}$	Log $C_2 = \frac{x_2}{x_1}\log C_1$	C_2	$\frac{C_1 - C_2}{C_1}$	% des.	Av. des.
100	20	1.3	377	333	0.883	1.148	14.0	0.30	30	28
200	10	1.0		261						
500	5	0.7	186	152	0.818	0.573	3.7	0.26	26	

It may be of interest here to compare the range of auxin used in this investigation to those used in studies on higher plants. Such a comparison is given in table XVIII.

Table XVIII. Comparison of ranges of auxin concentration.

Plant	Effective range of auxin conc. mg./liter	Author
Chlorella cult.	0.66 - 13.30 *	
Avena test	0.01 - 0.04	Went 1935
Pea test	1.00 - 10.00	Went 1935
Bean swellings	2.3 - 1165.0	Laibach 1935

* Taking into account the loss by sterilization

It is seen that the range of concentration used is comparable to that in the pea test; smaller than in the bean swellings and larger than in the Avena test. In this range we have seen that auxin, while it

promotes the growth of cell volume, has also begun to inhibit division. A lower concentration has been tried in agar medium and the results will be given in Chapter III.

The question arose as to whether Chlorella cells could produce auxin by themselves. Several tests were made on the media and on the cells themselves by chloroform extraction. The results were all negative. However, this fact does not necessarily mean that this alga is completely unable to synthesize auxin for it is possible that it produces auxin in very small quantities so that it is undetectable by the ordinary Avena test method without preliminary concentration. Van der Weij (1933) found the presence of auxin in the cell sap and cell wall of Valonia by concentrating the extract 10 - 100 times.

VIII. General discussion.

A. Cell enlargement.

Since the growth of Chlorella cells consists of cell division and cell enlargement, it seems profitable to discuss them separately.

The fact that auxin promotes cell enlargement in Chlorella has been definitely shown by the above experiments. It remains to consider the actual mechanism by which this takes place. It is well known that in higher plants the auxin causes an increase in the plasticity of the cell wall which favors the absorbtion of water and causes the cell to elongate. Increase of wall material and dry weight occurs in a later stage. The same mechanism probably exists in algae. Although there is no direct proof for this conception, the circumstantial evidence however supporting this view.

First, we have seen the general course of the growth cycle in both algae and higher plants is approximately the same and the chemical changes

involved in both cases are also strikingly similar (Pearsal and Loose 1935). Second, unlike fungi and bacteria, algae have the same cellulose wall as higher plants. In a recent paper the submicroscopic structure of the cell wall of Valonia has been investigated (Preston and Astbury 1937) and it was found that the micellar arrangement is comparable and traceable to that in wall of fibers in higher plants. Since the action of auxin is primarily on the micellar units (Bonner 1935) ~~and is even effective in dead fibers~~, there is very little doubt that it has shown the same activity here. Third, our data show that the same logarithmic relation exists between the auxin concentration and the cell size as in higher plants (section V). And finally, as will be shown later (Chapter II), auxin does not affect respiration in Chlorella. This indicates its action is primarily on the cell wall as in the case of the higher plants (Bonner 1936, Heyn 1931,1934).

Just as in higher plants auxin is not the only factor that limits the process of cell extension in algae. It has been shown that (section V) while in young cultures the cell size is strictly proportional to the logarithmic auxin concentration, in old cultures, especially at low auxin concentrations, this is no longer true. This fact suggests the presence of another factor which is unimportant in the young cultures but becomes limiting in the old ones. The exact nature of this factor is not known. That it is perhaps a question of food has been discussed (section V and section III). Food factor or factors have already been known in the higher plants (Went 1935). Whether these factors are of the same nature remains to be investigated.

Summing up, it may be concluded that as far as cell extension is concerned auxin has the same effect on Chlorella as in the higher plants.

It may prove to be a better object for the study of the auxin mechanism and growth, since it eliminates completely the question of transport (Went 1935) and because of its unicellular nature it furnishes a good object for the study of penetration and accumulation of auxin in cells.

B. Cell multiplication.

We have seen that while auxin promotes the growth in cell volume, in higher concentrations it inhibits cell division. ~~This results~~ the result of this is that the auxin cultures at a given age are always more dilute than the controls (section III) and contain a smaller number of cells (sectionVI). In this respect auxin differs from "bios" and B wuchsstoff, which stimulate cell division in yeast. The slowing down of the growth rate by auxin leads to a later saturation and a longer period of growth of the culture. In this sense it may be considered to have a rejuvenating effect. The inhibition of cell division with increasing concentration, and especially in high concentrations, is very well marked. In solid media um the effect is even more pronounced (see Chapter III). This phenomenon has lead Leonian (1937) to consider that auxin is an inhibiting, rather than a promoting, substance. His conclusion is, however, not true as we shall see in a later chapter (Chapter III).

Since very little is known as yet about the physiology of cell division, it seems premature to give any explanation of this inhibition by auxin. Nevertheless, there are some points in this connection worth mentioning. As will be shown later (Chapter II) in the case of young cultures the rate of photosynthesis of the auxin cells is lower than the controls and this lowering of photosynthesis is quantitatively related to a smaller chlorophyll content. Correlating these facts, it seems

reasonable to think of the inhibition process as consisting of the following steps:

auxin ⟶ chlorophyll ⟶ photosynthesis ⟶ cell division

In the first step just how auxin inhibits chlorophyll formation is unknown, and will not be discussed here (Chapter II). The second step, concerning the relation between chlorophyll and photosynthesis, has been worked out by Emerson (1929), Fleischer (1935) and the experiments presented in Chapter II. The last step, however, may consist of several short steps at present unknown. Since photosynthesis is the only source of food there in the cultures, it is not unreasonable to believe that it is related to cell multiplication through the food supply. Furthermore, recent works on cell division hormones, e.g. vitamine B, phytosterols, bean hormone, etc. have shown them to be largely found in the green parts of the plant, and so probably of photosynthetic origin. It is possible that a lower rate of photosynthesis decreases the supply of such factors or hormones, this then limiting cell division. A more direct connection between chlorophyll and the growth factors (vitamines) has been suggested in the literature (Schertz 1928). This, however, seems to the writer to be improbable.

In the older cultures the same thing appears to be true. In this case the auxin cultures have a higher photosynthetic activity than the controls and correspondingly they continue to increase in cell number while the control cultures have reached saturation and have practically ceased to multiply. The relation between chlorophyll and photosynthesis here is not so simple as in the young cultures and this will be discussed in a later chapter (Chapter II). It must be admitted in conclusion

that this scheme is of a tentative and hypothetical nature. It only gives a possible, perhaps quite probable, mechanism of auxin inhibition of cell division in the case of Chlorella. It also must be emphasized here that such inhibition, which appears only in comparatively high concentration of auxin, can therefore not be regarded as a normal attribute of auxin. Mistake in this regard has lead to quite erroneous conclusions.

IX. Summary.

(1) ~~A simple method was used to isolate~~ A single-cell clone of Chlorella vulgaris ~~and it~~ was cultured in inorganic liquid media with and without auxin (beta-indole-acetic acid). Cultures were grown for 7 to 15 days or longer. The various determinations were then made on them.

(2) The auxin cultures were found to be always more dilute than the cultures without auxin. They grew comparatively slower and therefore reached saturation later than the controls. This effect increased with the concentration of auxin. At very high auxin concentrations the cultures became injured and failed to grow.

(3) The dry weight of the cultures followed the same course as the volume concentration, i.e. it decreased with increasing concentration of auxin. The amount of dry matter per unit volume of cells was approximately constant.

(4) Measurement of cell diameters showed that auxin stimulated the growth in cell size. In young cultures the average volume of cells was directly proportional to the logarithm of the concentration of the auxin. In older cultures this was not so apparent owing to the presence of another factor.

(5) The number of cells per unit volume of the culture decreased with increasing auxin concentration, indicating that auxin inhibited or slowed up the rate of cell division.

(6) Under normal conditions no appreciable decrease of auxin could be found in the cultures for 7 - 15 days. No detectable amount of auxin was produced by Chlorella cells themselves.

(7) Discussions were given concerning the mechanism of growth of Chlorella and on the effects of auxin. Conclusions were reached that (a) the same mechanism exists in Chlorella as in higher plants in the process of cell enlargement brought about by auxin and (b) inhibition of cell division by auxin in high concentrations could be explained by a decreased amount of chlorophyll and photosynthesis.

II

Effect of Auxin on the Pigment Contents, Respiration and Photosynthesis of Chlorella vulgaris.

I. Introduction.

It has been shown in the foregoing chapter that the effect of heteroauxin on Chlorella vulgaris is two-fold: In the concentrations used (0.001 - 0.020 mg./c.c.) it stimulates the growth of cell size but inhibits multiplication of cells by division. It was the purpose of the following experiments to find out whether the effects could be correlated with any change in the metabolic activities.

Four experiments were made in this investigation, the material used being Chlorella cells cultured in experiments 4, 5, 6, and 8 of the last chapter. Detailed information on the method of culturing and the results of growth measurement are found in section II, Chapter I. Determinations were made of the pigment content, respiration and photosynthesis. The method used and the results obtained will be described separately in the following sections.

II. Determination of chlorophyll concentration.

The pigments present in the chloroplasts of the cells comprise the chlorophylls, carotin, and xanthophyll. The concentration of chlorophyll per unit volume of cells was determined by washing a known volume of cells with hot water, and extracting two or more times with methyl alcohol until no color was left in the cells. The total extract was made up to a known volume (10, 25, or 35 c.c.) in volumetric flasks. The extinction coefficient ϵ_{N_e} of a sample of the solution containing all the pigments was determined for the wave-length 6598 Å. from a neon tube.

This point has been found to lie completely outside the absorption curve for the carotenoids and is approximately at the peak of the chlorophyll absorption band in the red (Emerson 1929). The value ϵ_{NE} was then transformed into absolute units, i.e. moles of chlorophyll per $mm.^3$ of cells by the following expression (Emerson and Arnold 1932):

$$n = \frac{\epsilon_{Ne}}{v\epsilon'_{Ne}m} X \frac{10\ V}{1000^2}$$

where n is the number of moles of chlorophyll per $mm.^3$ of cells.
ϵ'_{Ne} is the extinction coefficient for 10 mg. of chlorophyll/liter (0.476)
m is the molecular weight of chlorophyll (906.6)
v is the $mm.^3$ of cells used in the extract.
V is the volume of the methyl alcohol solution.
and ϵ_{Ne} is the observed extinction coefficient.

Table XIX. Determinations of chlorophyll. (Exper. 5, March 14, 1937).

Culture ---	1	3	4	5	6
Auxin conc.	0	0.005	0.010	0.020	0.040
#flask ----	163	325	326	927	3
C.c. CH_3OH	25	25	25	35	10
$Mm.^3$ of cells	86.6	85.6	97.5	96.4	29.7
α_1	61.5	62.2	64.2	60.3	50.3
α_2	38.0	37.5	34.7	39.3	50.0
ϵ_{Ne}	0.3724	0.3930	0.4753	0.3308	0.0046
n x 10^{+9}	2.49	2.69	2.82	2.78	0.04
n'/cell x 10^{+12}	1.52	1.92	2.12	2.42	0.084

Table XIX gives an illustration of a chlorophyll determination and table XX gives the results of four experiments, in which the concentration of chlorophyll is expressed in moles per $mm.^3$ cells and per single cell, together with the average volume of the cells

for comparison. Since in experiment 4 the cell number was not measured only moles of chlorophyll per $mm.^3$ of cells is given.

Table XX. Chlorophyll content.

Exper. -------		6			8			5			4
Days ---------		7			15			16			15
Culture No.	Auxin conc.	$n/mm.^3$ 10^9	$n/cell$ 10^{15} *	cell vol.	$n/mm.^3$ 10^9	$n/cell$ 10^{15}	cell vol.	$n/mm.^3$ 10^9	$n/cell$ 10^{15} *	cell vol.	$n/mm.^3$ 10^9
1	0	2.97	1.19	151.9	2.28	0.87	140.0	2.49	1.52	142.3	1.73
2	0.001	3.70	1.64	248.5	2.13	0.81	132.9	-	-	-	1.76
3	0.005	3.33	3.47	533.2	2.76	1.15	205.0	2.69	1.92	210.7	2.46
4	0.010	2.61	2.61	572.2	3.03	1.63	266.9	2.82	2.12	306.8	2.24
5	0.020	1.30	1.48	689.5	-	-	-	2.78	2.42	452.4	-
6	0.040	-	-	-	-	-	-	0.04	-	-	0

* Owing to the innacuracies of cell counting, these values are not ~~to be taken too seriously~~ very dependable (see Chapter I, section 6).

It is seen that in the older cultures (experiments 5 and 8) the amount of chlorophyll per $mm.^3$ of cells is about the same in both auxin and control cultures. This means that the amount of chlorophyll per unit quantity of cell material or protoplasm is constant, if we consider the volume concentration as a measure of the cell material. The slightly higher values for the auxin cultures are due to the fact that the cells in these cultures are larger and are packed tighter in the determinatiom of the volume concentrations by centrifuging (see Chapter I, section 6). The same fact can also be observed in the values given for the amount of chlorophyll per single cell. These values are higher, the higher the concentration of auxin, i.e. the larger the volume of a single cell. Therefore under these conditions a ~~close relation~~ parallelism exists between the

development of chlorophyll and the growth in cell volume.

In the young cultures this is, however, not strictly true. While in low auxin concentration cultures (cultures 1, 2, and 3) the chlorophyll content increases with cell volume, in the high auxin concentration cultures it falls behind the cell volume. The explanation for this is perhaps as follows: First, the growth in cell volume in the presence of a high auxin concentration is so much quicker than the formation of chlorophyll that the latter is left behind; second, that in high concentrations of auxin, the chlorophyll formation may be actually retarded, as we have shown in very high auxin concentration (0.040 mg./c.c.) the cells fail completely to develop any chlorophyll and become brownish red in color. It is possible that in comparatively high concentrations auxin may in some way interfere with the absorption of such ions as Mg, Fe, or NO_3, which have been found to be very important in the chlorophyll development in Chlorella (Fleischer 1935, Emerson 1929). On the other hand it may be connected with some destructive mechanism of chlorophyll, the nature of which is not yet well known. It is important to emphasize here that the destruction or inhibition of development of chlorophyll is by no means a normal accompaniment of auxin, as the concentration concerned with here is quite high.

On account of the low chlorophyll content, the young auxin cultures have a lower photosynthetic rate than the corresponding controls (section VI) and this may consequently be the cause of the slow rate of growth in these cultures (Chapter I).

IV. Determination of the carotenoids.

The same methyl alcohol solutions were used in the carotenoid determinations. The procedure was also the same except a different wave-length 4359.8 Å. from a mercury source was used. At this wave-length the carotenoids have approximately their maximum absorption and the chlorophyll absorbs about the same amount as at the other wave-length. Therefore, by comparing the extinction coefficients of the solution at the two wave-lengths the amount of carotenoids relative to chlorophyll can be obtained. The values $\frac{\epsilon_{Hg}}{\epsilon_{NE}}$ are given in the following table. They can be translated into absolute units when the extinction coefficient for a standard solution is known for this new wave-length.

Table XXI. Carotenoids in the cells.

Exper. --------		4			5		
Days ----------		15			16		
Culture No.	Auxin conc.	ϵ_{Hg}	ϵ_{NE}	$\frac{\epsilon_{Hg}}{\epsilon_{NE}}$	ϵ_{Hg}	ϵ_{NE}	$\frac{\epsilon_{Hg}}{\epsilon_{NE}}$
1	0	0.9869	0.2684	3.7	1.1859	0.3724	3.4
2	0.001	1.2035	0.2992	4.0	-	-	-
3	0.005	0.8408	0.2407	3.5	1.2560	0.3930	3.2
4	0.010	0.9506	0.2570	3.7	1.4193	0.4753	3.0
5	0.020	-	-	-	1.1238	0.3308	3.4
6	0.040	0.1771	1.9924	-	0.4179	0.0046	90.9

From the table it is seen that the value $\frac{\epsilon_{Hg}}{\epsilon_{NE}}$ is approximately a constant for the different cultures. Since the chlorophyll content of these cultures (experiments 4 and 5) has been shown in the above paragraph to be nearly constant, the carotenoid contents of

of these cultures are also approximately the same. Unfortunately no determination of the carotenoids had been made in the young cultures (experiment 6) where as shown above, the chlorophyll concentration varied with that of the auxin.

In the cultures of highest auxin concentration (0.040 mg./c.c., culture 6, experiment 5) the ratio $\frac{\epsilon_{HE}}{\epsilon_{NE}}$ is abnormally high. This is due to the extremely small amount of chlorophyll (table XX). The cells have a brownish red color to the naked eye. The red pigments in these cells are probably not all carotenoids. They are not easily extracted with methyl alcohol or petroleum ether, and do not give the microchemical tests of the carotins (Kohl 1902). They are probably decomposition products of some cell constituents, as the cells are apparently disintegrating.

V. Respiration.

Respiration was measured manometrically along with photosynthesis as described by Warburg (1926) and Emerson (1929). About 10 $mm.^3$ of cells were taken for each culture. They were washed and suspended in Knop's solution previously saturated with air containing 5 % CO_2. 7 c.c. of this suspension was placed in each vessel. A current of 5 % CO_2 in air was allowed to pass through the vessel and the manometer for two minutes. The vessels were then placed in a thermostat at 25°C. where they were constantly shaken by means of an electric motor. The respiration readings were made in darkness at intervals of 30 minutes. The rate of respiration, R, in $mm.^3$ of O_2 absorbed per $mm.^3$ of cells per hour was calculated from the change of pressure (Δh), the vessel constant (K_1) and the number of $mm.^3$ of cells used by the relation:

$$R = \frac{K_1 \Delta h}{m} \times \frac{60}{30}$$

A typical determination of respiration is shown as follows:

Table XXII. Respiration measurements. (Exper. 5, March 12, 1937).

Culture No.	Auxin conc.	$mm.^3$ cells used	no. of man. vessel.	K_1	Change in pressure.	R $mm.^3 O_2/mm.^3$ cells/hr
1	0	9.1	7	1.27	2.5	0.70
2	0.005	8.99	8	0.73	4.8	0.78
4	0.010	10.92	9	1.37	3.2	0.80
5	0.020	11.81	10	0.74	5.8	0.73
6	0.040	17.36	12	1.34	0.5	0.77

The next table gives the results of four experiments:

Table XXIII. Summary of experiments on respiration.

Exper. -----		6	4	8	5
Days -------		7	15	15	16
Culture No.	Auxin conc.	R ($mm.^3$ of O_2/ $mm.^3$ of cells/hr.)			
1	0	0.86	0.70	0.81	0.70
2	0.001	1.14	0.42	0.87	-
3	0.005	0.82	0.73	0.94	0.78
4	0.010	0.95	0.30	0.92	0.80
5	0.020	0.84	-	-	0.73
6	0.040	-	0.03	-	0.77

It is quite evident that heteroauxin ~~does not affect the rate of photosynthesis but~~ has no influence on respiration. The same phenomenon has been observed in Avena coleotiles (Bonner 1936). This gives another indirect piece of evidence for the same mechanism of cell enlargement in Chlorella and in higher plants (see Chapter I).

VI. Photosynthesis.

The light source used in photosynthesis measurements consisted of a series of four to seven 100-watt internally frosted incandescent lamps placed below the thermostat. This arrangement has been found to give a saturating (non-limiting) light intensity. Readings were taken at five minute intervals and results were calculated in the same manner as for respiration.

The results of three experiments on photosynthesis are given in tables XXIV to XXVI, in which column 8 gives the rate of photosynthesis in $mm.^3$ O_2 produced per $mm.^3$ of cells per hour corrected for respiration. Column 9 gives the same quantity in $mm.^3 \times 10^{-6}$ of O_2 per cell per hour.

Table XXIV. Photosynthesis. (Exper. 5, 15-day culture, Feb. 24 - March12) Temp. 25°C. Illumination 7 x 100 watts.

Cult. No.	Auxin conc.	$mm.^3$ of cells.	No. of vessel	K_1	Change in pressure	Q_{O_2} mm.	Q_{O_2} + R mm.	Q_{O_2} + R/cell mm x 10^{-6}
1	0	9.1	7	1.27	2.9	4.86	5.56	3.39
2	0.005	8.99	8	0.73	6.1	5.94	6.72	4.80
4	0.010	10.92	9	1.37	5.6	8.44	9.24	6.95
5	0.020	11.81	10	0.74	22.8	17.17	17.94	15.74
6	0.040	17.36	12	1.34	-0.1	0	0.77	-

Table XXV. Photosynthesis. (Exper. 8, 15-day culture, Mar. 29 - Apr. 13). Temp. 25°C. Illumination 6 x 100 watts.

Cult. No.	Auxin conc.	$mm.^3$ of cells.	No. of vessel	K_1	Change of pressure	Q_{O_2} mm.	Q_{O_2} + R mm.	Q_{O_2} + R/cell mm. x 10^{-6}
1	0	10.29	7	1.27	2.49	3.69	4.50	1.72
2	0.001	9.96	9	1.37	1.97	3.26	4.13	1.58
3	0.005	9.52	10	0.74	6.34	5.91	6.85	2.85
4	0.010	9.55	12	1.34	4.45	7.50	8.42	4.53

Unlike respiration, the role of photosynthesis is markedly affected by auxin. In the older cultures especially (experiment 8 and 5) the higher the auxin concentration the higher is the rate of photosynthesis. In the young cultures (experiment 6) the same thing is true for the lower auxin concentrations (0.001 - 0.005 mg./c.c.) but in the higher concentrations (0.010 - 0.020 mg./c.c.) the photosynthetic rate drops off again, although still above the controls.

Table XXVI. Photosynthesis. (Exper. 6, 7-day culture, March 18, 1937). Temp. 25°C. Illumination 7 x 100 watts.

Cult. No.	Auxin conc.	$mm.^3$ of cells.	no. of vessel	K_1	Change in pressure.	Q_{O_2} mm.	Q_{O_2} + R mm.	Q_{O_2} + R/cell mm.
1	0	8.54	7	1.27	10.9	19.4	20.26	8.14
2	0.001	9.06	8	1.10	17.3	25.2	26.34	11.66
3	0.005	9.09	9	1.37	9.5	17.2	18.02	18.77
4	0.010	8.40	10	0.74	10.6	11.2	12.15	12.15
5	0.020	8.46	11	0.99	5.3	7.5	8.34	9.48

If the corresponding cultures of two different ages be compared, it will be seen that the photosynthetic activity tends to decrease with age, although the decrease in the auxin cultures is smaller than in the controls. In this respect it appears that the auxin cultures are physiologically younger than the controls of the same chronological age (see Chapter I).

To find out whether auxin has a direct effect on photosynthesis the following experiment was undertaken. Three samples of cells were taken from a culture without auxin, washed, and suspended in (1) CO_2-Knop's, (2) CO_2-Knop's plus auxin to give a concentration of 0.005 mg./c.c. and (3) CO_2-Knop's plus auxin to give a concentration of 0.010 mg./c.c.

The rate of photosynthesis of these three samples was measured manometrically at 1, 7, 25, and 32 hours after they were put in the auxin solutions. The results are given in the following table:

Table XXVII. Immediate effect of auxin on photosynthesis and respiration. (Exper. 7, March 31 - April 2, 1937)

Auxin conc.	mm^3 of cells.	No. of vessel	K_1	Photosynthetic Q_{O_2} 1	7	25	32 hours	Respiration R 1	7 hours
0	9.45	8	0.73	15.2	19.1	19.3	21.5	0.49	0.51
0.005	9.45	10	0.74	13.6	15.7	14.7	17.2	0.46	0.55
0.010	9.45	11	0.99	13.7	16.0	14.4	16.0	0.43	0.54

No significant difference in the photosynthetic rates is observed, the slight difference in the Q_{O_2} ~~was~~ is probably due to the error in the measurement and preparation of the cell samples. From this it may be concluded that this effect of auxin on photosynthesis is not direct but is due perhaps to some change in the cell during a comparatively long period of cultivation.

VII. The relation between photosynthesis, chlorophyll, and surface.

Emerson (1929) and Fleischer (1935) have both shown that in young cells (5 to 7 days old) cultured in Fe or N deficient media a linear relation exists between the rate of photosynthesis and the concentration of ~~the~~ chlorophyll. The same thing is observed here in the 7-day cultures (experiment 6). Table XXVIII shows that the ratio of the photosynthetic rate per cell to the chlorophyll per cell is approximately constant for the different cultures, with and without auxin. Single cells, instead of mm^3 of cells, are used here as a unit because it is I believe that volume concentration or mm^3 of cells determined by centrifuging is not a very satisfactory ~~quantity~~ measure for cells of different sizes

(Chapter I). The inaccuracy of cell counting does not affect the result here, for in the calculation of Q/chl from Q/cell and chl/cell it is cancelled out.

In the last column of table XXVIII the average cell surface is given. It can be seen that cell surface has nothing to do with the photosynthesis in these young cultures. The same thing has been suggested by Fleischer (1935).

Table XXVIII. Photosynthesis and chlorophyll in young cultures. (Exper. 6, 7-day culture)

Culture No.	Auxin conc.	O_2 produced per cell per hour. $mm^3 \times 10^{-6}$	Chl/cell $n \times 10^{-15}$	O_2 produced per mole of chl. per hour. $mm^3 \times 10^{-9}$	Cell surface $mm^2 \times 10^{-4}$
1	0	8.14	1.19	6.8	1.263
2	0.001	11.66	1.64	7.1	1.912
3	0.005	18.77	3.47	5.4	3.181
4	0.010	12.15	2.61	4.7	3.332
5	0.020	9.48	1.48	6.4	3.775

In the old cultures (15 - 16 days) the linear relation between the rate of photosynthesis and chlorophyll content no longer holds. Column 5 of table XXIX shows that the ratio Q (cell)/chl (cell) is not constant but increasing steadily with the increase in cell surface (column 6) so that if Q (cell)/ chl (cell) is plotted against the average cell surface a straight line is obtained (figure 6).

One remarkable thing is seen in this treatment. In the two experiments performed at different times the individual quantities (photosynthesis, chlorophyll and cell surface) varied considerably (see columns 3, 4, and 6), yet the relation Q/chl to cell surface falls exactly on the same straight line. This fact seems to exclude any

possibility that the relation is an accidental one.

Table XXIX. Relation between photosynthesis, chlorophyll and cell surface. (Mature cultures.)

	Cult. No.	Auxin conc.	O_2 produced per cell per hour. 10^{-6}	Chl/cell n x 10^{-15}	Q (cell) / chl (cell)	Cell surf. mm^2 x 10^{-4}	K x 10^{-5}
Exper. 5	1	0	3.39	1.52	2.23	1.319	1.7
	3	0.005	4.80	1.92	2.50	1.711	1.5
	4	0.010	6.95	2.12	3.28	2.201	1.5
	5	0.020	15.74	2.44	6.45	2.850	(2.3)
Exper. 8	1	0	1.72	0.87	1.98	1.304	1.5
	2	0.001	1.58	0.81	1.94	1.260	1.5
	3	0.005	2.85	1.15	2.48	1.682	1.5
	4	0.010	4.53	1.63	2.78	2.005	1.4

(experiment 5 was 16-day culture, 8 was 15-day culture).

The only culture in which Q/chl to surface does not fall in line is culture 5 in experiment 5. This is understandable if we examine its Q/chl value which is much larger than the rest and is comparable to the values obtained in the young cultures (column 5, table XXVIII). The culture is, as before said, physiologically young (Chapter I) and therefore not comparable with the rest.

To summarize, the results of the present experiments indicate that at least three internal factors are involved here in photosynthesis: age, chlorophyll, and cell surface. In young cultures the rate of photosynthesis depends upon the concentration of chlorophyll. In old cultures it depends upon both chlorophyll concentration and the extent of cell surface. The effect of auxin is indirect through the increase in cell surface.

The literature on this phase of photosynthesis is rather limited.

Practically nothing is yet known about the age factor. The relation between chlorophyll and photosynthesis has recently been discussed by Emerson (1936) who came to the conclusion that it is not the chlorophyll that actually limits the rate but a substance combining with CO_2 and whose concentration may vary linearly with the chlorophyll. The importance of surface in photosynthesis has been repeatedly suggested although without any evidence (Warburg 1928, Baly 1927). From the results obtained here it seems that photosynthesis is related somehow to a surface reaction or a factor which varies with the extent of the surface of the cell.

Recently it has been shown by Emerson (1935) that Chlorella cells, exposed to strong light for several hours, show a large increase in the rate of photosynthesis without any increase in the chlorophyll content. Since no measurement of the cell diameter is available detailed treatment can not be given. However, it can be shown to fit quite well with the present scheme if a suitable increase in surface is assumed. Experiments of this nature, it seems to the writer, may lead to some elucidation of the complex process of photosynthesis.

VIII. Summary.

(1) Chlorella cells cultivated with and without auxin were used. Their growth characters were described in chapter I.

(2) Determinations were made of the chlorophyll content, carotenoid content, respiration and photosynthesis.

(3) Chlorophyll was found to vary directly with the quantity of cell material. In young cultures high auxin concentrations seemed to retard chlorophyll formation.

(4) Carotenoids varied in the same manner as the chlorophyll.

(5) Respiration was not affected by auxin in all concentrations, a condition comparable to that in the higher plants.

(6) Photosynthesis was markedly affected by auxin. In old cultures photosynthetic activity increased with auxin concentration. In young cultures the same was true for low auxin concentrations, but in high auxin concentrations the rate of photosynthesis drops off, due to a low content of chlorophyll.

(7) In young cultures photosynthesis was shown to be a linear function of chlorophyll concentration, and in old cultures photosynthesis was a linear function of the product of chlorophyll concentration and the cell surface.

III

Effect of Auxin on the Growth of Chlorella vulgaris in Agar Medium.

I. Introduction.

The following experiment is of a preliminary nature. Its purpose is three-fold.

(1) To find the effect of heteroauxin on Chlorella on solid media.

(2) To test the activities of the substances chemically related to auxin.

(3) To test the activity of vitamine B_1 on Chlorella.

Special interest was given to the action of heteroauxin itself. The reasons were: first, to compare the effect of heteroauxin on Chlorella on solid media with the results obtained in liquid cultures (Chapter I), and second, to repeat more carefully the results of Leonian (1937) on the inhibitory action of heteroauxin on a very similar species of algae (Chlorella pyrenoidosa) on solid media.

Previous experiments along these lines of study are varied and scattered. They will be discussed separately in a later section.

II. Materials and method.

The pure clone (A15a) of Chlorella vulgaris isolated in the former experiments (Chapter I) was used. The agar media were made according to the modified Benecke formula, described in Chapter I, section II. Fifty-two clean and dry petri dishes were used, 20 c.c. of the medium being poured into each. They were sterilized in an autoclave at eighteen pounds pressure for twenty minutes.

Ten stock solutions of the different substances were made. They had the following concentrations:

Table XXX. Concentrations of various test substances used.

No. of stock solution.	Substance	Conc. mg./c.c.
1	β-indole acetic acid	0.2
2	" " " "	0.02
3	" " " "	0.002
4	benzoic acid	10.0
5	phenyl-acetic acid	1.0
6	phenyl-propionic acid	10.0
7	phenyl-butyric acid	10.0
8	cis-cinnamic acid	1.0
9	trans-cinnamic acid	10.0
10	vitamine B_1	0.01

Both vitamine B_1 and heteroauxin were obtained as pure crystals from Merck. The rest of the chemicals were synthesized in the chemical laboratory of this institute.

The stock solutions were separately sterilized, cooled, and added to the sterile media before the agar began to set. Each medium was then inoculated with one c.c. of a dilute sterile solution of Chlorella. Each was throughly shaken to ensure a uniform distribution of cells. The whole operation was carefully carried out under aseptic conditions. Six cultures were used as controls and all the other cultures were made in duplicate.

The culture dishes were then placed upside down on large pieces of filter paper which served as a white background. They were covered

first with a transparent sheet of cellophane and then with a glass hood to protect them from contamination. The whole set was put under a Northern window.

III. Results.

The green color was first discernible about one week after the cultures were inoculated. The growth was comparatively slow on account of the prevailing low temperature during the experiments. After a period of 38 days the cultures were removed and studied. Since no satisfactorily quantitative method of measurement could be used here, they were compared on the gross appearance of the culture, i.e. the area and the thickness of the growth. As the control cultures had the largest areas and the thinnest growth, all the other cultures could be easily compared with them. Their areas and thicknesses were rated in artificial units referring to the controls as unity. The product, area X thickness, was then used as an index of the extent of the growth. A substance was considered to have a stimulating effect (+) when A X T was greater than 1, and an inhibiting (-) effect when A X T was less than 1. Small differences (less than 0.5) from the control were not counted. The results are summarized in table XXXI and photographs of the cultures are given in plates 1 - 3, figures A - E. (See next page for table XXXI.)

Microscopical examinations were made of the cultures. It was found that the thinnest cultures (controls) consisted of small, widely scattered colonies, each colony comprised of a cluster of about 8 to 128 cells; the thicker cultures (II_a) contained larger colonies of over 200 cells generally; and the thickest cultures (II_b, etc.) con-

follows p. 39

Table XXXI. Heteroauxin & Auxin-like substances and growth of Chlorella vulgaris on agar media containing these substances. (Exper. 9, Feb. 6, 1937).

Culture No.	Substance tested	Conc. mg./c.c.	No. of cult.	Remarks on growth characteristics. area	thickness	A x T	Activity Chlorella	Pea
I	control	0	6	1	1	1		
II a	heteroauxin	0.00001	2	½	3	1.5	?	0
b	"	0.0001	2	1	4	4	+	0
c	"	0.001	2	1	4	4	+	+
d	"	0.01	2	¼	4	1	0	+
e	"	0.04	2	0	0	0	-	+
III a	benzoic acid	0.05	2	¼	6	1.5	?	0
b	"	0.1	2	1/6	4	0.7	-	0
c	"	0.5	2	0	0	0	-	0
IV a	phenyl-acetic	0.005	2	¼	6	1.5	?	+
b	"	0.01	2	¼	4	1	0	+
c	"	0.05	2	0	0	0	-	+
V a	phenyl-proprionic	0.05	2	0-0.1	0-1	0-0.1	-	+
b	"	0.1	2	0	0	0	-	+
c	"	0.5	2	0	0	0	-	+
VI a	phenyl-butyric	0.05	2	¼	3	0.6	?	0
b	"	0.5	2	0-0.1	0-3	0-0.3	-	0
VII a	cis-cinnamic	0.005	2	1	2	2	+	+
b	"	0.01	2	1	1	1	0	+
c	"	0.05	2	½	1	0.5	-	+
VIII a	trans-cinnamic	0.05	2	¼	5	1.3	?	0
b	"	0.5	1	0	0	0	-	0
IX a	vitamine B_1	0.0005	2	1/3	6	2	+	
b	"	0.001	2	1/3	5	1.7	+	
c	"	0.005	1	1/3	5	1.7	+	

taining closely packed clusters of over 1000 cells each. These facts are shown in plates 3 and 4, figures F to H.

Before going into a discussion several things should be first mentioned. First, the observational error was much smaller than it might appear to be. The differences between the cultures (especially of series I, II, and IX) were so striking that very much the same rating was given by several observers consulted. Second, the variation between the duplicate cultures was again very small so that it was possible to give one rating for them. So far only two exceptions were observed; these were the cultures V_a and VI_b where one culture of the duplicate gave a very small growth while the other culture failed to develop completely.

Other possible sources of error may be cited: slight differences (1) in the amount of inoculum, (2) in the osmotic concentration of the media due to the addition of the test solutions (0.1 - 1.0 c.c. in the 20 c.c.), and (3) in light conditions due to differences in position. All these errors were small compared to that of observation and were therefore negligible. The possibility of infection was excluded here as it was found at the end of the experiment only one culture out of the fifty-two showed a faint sign of fungal growth (II_e).

IV. Conclusions and discussion.

(1) Heteroauxin.

Table XXXI and plate 1A show that heteroauxin (beta-indole acetic acid) is active in stimulating growth in very low concentrations (0.00001 - 0.0001 mg./ c.c.). At higher concentrations (0.01 mg./c.c.) its stimulating effect disappeared and at still higher concentrations (0.04 mg./c.c.) it inhibited growth completely.

The optimum range of concentration was therefore lower than that found for cell enlargement in liquid cultures (0.001 - 0.02 mg./c.c.). This suggests that a different phase of growth, perhaps cell division, was mainly concerned here. This suggestion was borne out by microscopic examinations, which show that the cell clusters derived presumably from single cells contained a larger number of cells in the auxin cultures than in the controls (plates 3 and 4, figures F to H).

In the case of liquid cultures (Chapter I) the lowest auxin concentration (culture 2, 0.001 mg./c.c.) compared with the controls always had the same or even a higher volume concentration. They had approximately the same number of cells per c.c. (Chapter I). A similar (or slightly better) effect was observed here. Also the highest concentration of auxin produced the same effect in both liquid and solid media, i.e. complete inhibition of growth. It seems, therefore, that auxin has a dual effect on Chlorella: in low concentrations it stimulates cell division, while in high concentrations it retards division but promotes the increase in volume of the cell.

In the last two columns of table XXXI the activity of heteroauxin on Chlorella and in the pea test were compared. It can be seen that the optimum concentration for promoting Chlorella growth is lower than that for the pea test. In the latter case, owing to the presence of cut surfaces, the destruction is probably high. A higher concentration of auxin (0.04 mg./c.c.), while producing no injury to the pea seedlings, is already inhibitory to Chlorella growth. This fact indicates that the optimal range of concentration for Chlorella is comparatively narrow, and that care must be exercised in the choice of concentrations to be used (see next section).

A recent paper by Leonian and Lilly (1937) is of interest. These authors cultured seven species of algae, including two Chlorella (C. pyrenoidosa and C. viscosa), on dextrose agar plus 10^{-6} to 10^{-5} parts (0.001 - 0.01 mg./c.c.) of heteroauxin. There results showed that in the low concentrations the growth was the same in auxin and control cultures, but in the higher concentrations the growth was retarded by auxin. While the concentration of auxin used by Leonian and Lilly was comparable to those in cultures II_c and II_d of our experiments (table XXXI), their results were nearer to those of cultures II_d and II_e. This discrepancy was perhaps due to the difference in the sensitivity to auxin of the different species or to a difference in experimental technique or the source and purity of the heteroauxin which they failed to mention. Unfortunately Leonian and Lilly did not use any lower concentrations of heteroauxin. This was probably the reason for their failure to obtain the stimulating effect. Indeed, the concentrations of heteroauxin they used appear to be very low ~~to the ordinary mind~~, yet it must be remembered that one-hundredth of their lowest concentration (0.00001 mg./c.c.) has been shown to be effective to promote growth in some plants (Avena). It seems rather hasty, therefore, for them to draw from limited data the conclusion on the general ~~nature~~ action of heteroauxin.

(2) Auxin-like substances.

The concentrations of the substances used in these experiments were chosen on the basis of their activities in the pea test. They were apparently too high for Chlorella for the reasons given above. No definite conclusion can therefore be drawn from these results. Only a few remarks can be made. At higher concentrations all the

substances were inhibitory. At lower concentrations slightly better growth was observed in cis-cinnamic acid, and less in phenyl-acetic acid, benzoic acid, and trans-cinnamic acid. Inhibition was observed in phenyl-butyric acid and phenyl-propionic acid. Recently Elliot (1936) reported an experiment on protozoa. He used heteroauxin and also gamma-3-indole butyric acid and beta-3-indole propionic acid and found all the compounds stimulated the growth of the chlorophylleus forms of the protozoa.

(3) Vitamine B_1.

Noticeable increase in growth was observed in cultures containing crystalline vitamine B_1 (0.0005 - 0.005 mg./c.c.) (table XXXI, IX a,b,and c, and plate 3E). Microscopic examination revealed that the increase in growth was probably due to an increase in cell division (plate 4H).

The growth stimulating effect of vitamines has been reported recently for a number of plants, e.g. fungus (Schopfer 1936) and the root of higher plants (Bonner 1937). In algae Leonian (1937) found no stimulation. The concentration he used was high (10^{-5} parts or 0.01 mg./c.c.). The results were therefore not comparable with those presented here.

V. Summary.

(1) Chlorella vulgaris was grown in agar medium with the addition of heteroauxin, vitamine B_1, and several other compounds chemically related to heteroauxin.

(2) Heteroauxin was found to stimulate growth at very low concentrations (0.0001 - 0.001 mg./c.c.) and inhibit growth at higher concentrations (0.04 mg./c.c.). The increase in growth was perhaps due to an increase in cell division.

(3) Vitamine B_1 was found to stimulate growth in low concentrations (0.0005 - 0.005 mg./c.c.).

(4) No definite conclusion can be drawn concerning the activities of the various auxin-like compounds because the concentration ranges that were chosen were comparatively high.

IV

Diaphototropic Movement of the Leaves of Malva neglecta

I. Introduction.

The fact that the leaves of some plants orientate themselves perpendicularly to the incident light has been known for a long time. Among the first who observed the phenomenon were Bonnet (1754) and Dutrochet (1833). Later it was studied by Frank (1870) who gave it the name "Transverse heliotropism". Frank thought that light was the causative agent for the movement but de Vries believed that a variety of factors were responsible, e.g. geotropism, epinasty, and hyponasty and weight of the leaves. The question was settled in favor of Frank's hypothesis by the work of Darwin (1881) and Vöchting (1888). Darwin also proposed the name "Diaphototropism" which has since been in general use.

In the beginning of the present century attention was directed to the question of the perception of light by leaves. Haberlandt (1905) founded the lens-action hypothesis which was later disproved by the work of Kniep.

Diaphototropic movements can be observed in the leaves of many plants, chiefly Leguminosae and in flower heads (du Buy and Nuernbergk 1932) such as the sunflower. They are often mentioned in connection with ecological considerations. The physiological side of the question has not been studied except by Vöchting and Denecke (1924). As far as is known by the writer the movement has not been carefully analyzed and its mechanism remains obscure.

In the present study the leaves of Malva neglecta were used.

Beside exhibiting the daily diaphototropic movement, these leaves also showed a number of other movements such as epinasty, geotropism and phototropisms of the peticle and sometimes sleeping movements. To avoid confusion it seems necessary at the outset to define the diaphototropic movement: it is the orientation of leaves in such a way that the upper faces of the laminae place themselves perpendicular to the incident light. In fact, the leaves are so sensitive that they, as described by Bonnet, "suivent en quelque sorte le cours du soleil: en sorte que le matin ces feuilles regardent le levant; vers le milieu du jour, le midi; le soir, le couchant" (Vöchting 1888).

It is aimed in the present paper: (1) to determine the directive action of light, (2) to differentiate the organs of perception and reaction, and (3) to discover the mechanism of the movement.

II. Effect of light on the movement.

A. Observations under normal light conditions.

Young Malva plants grown in the field were transplanted into the greenhouse. Two or four weeks after transplantation, when the plant had three or four leaves, experiments were started. The movements of the leaves were photographed with a Zeiss cinematographic camera which automatically made an exposure every six minutes for twenty four hours. The film was then developed, projected, and the measurements were made.

Figure 7 shows the results of such an experiment. The abscissa represents the time in hours starting from midnight. The ordinates give the degrees in angles that the leaf planes made with the horizontal, the angle being zero when the leaf faced upward, negative when it faced east and positive when it faced west. The three curves

give the movements of three leaves during the whole day. It is clear from the curves that during the night all the leaves faced east and remained in that position until dawn. As the sun rose they turned upward gradually reaching a horizontal position at noon. They then turned further toward the west with the sun and after the sun had set, gradually turned back so that they faced east before midnight. The straight line represents the change of the hour angle of the sun calculated on the basis that the sun moves 15° an hour from 6 am. to 6 pm. It is seen that the leaves oriented ~~teted~~ themselves towards the sun, always keeping their leaf blades perpendicular to the sun's rays. The oldest leaf (lowest curve) however was less sensitive.

The fact that the leaves returned to face east in the night is particularly interesting. This phenomenon has never been reported before although it appears quite important in understanding the mechanism of the whole movement. It can not be considered as a sleeping movement since it bears no relation to the insertion of the leaves and depends only upon the geographic directions. Thus leaves joined to the stem on the east side of the plant bend adaxially and leaves on the north and south sides turn sideways.

The returning of the leaves after sunset is autonomous and independent of external conditions. Several experiments have been made by turning the plants through 180° just before sun down so that the leaves were now facing east instead of west. After several hours it was observed that the leaves, instead of staying where they were, had turned toward the west, i.e. their original moving position with respect to the plant. In other experiments the plants were turned 90° and the

same result was obtained. This character lasted for several days before the plant was "adapted" to its new direction of movement. A condition, however, has been found necessary for this returning movement: the plants must have "morning light", i.e. they must be placed in such a position as to receive direct light from the sun in the early morning. If this condition was not satisfied, e.g. if the plants were grown against an eastern wall where they could not get direct sunlight until noon, then in the night their leaves would not turn toward the east but drop down abaxially as in ordinary epinastic movement.

B. Observations in diffuse light.

To find out whether sunlight has a directive influence on the movement a similar experiment was performed on a rainy day when the sky was overcast and the position of the sun could not be determined. The results are given in figure 8. Throughout the whole day the leaves remained in a more or less horizontal position. In the morning and in the night they turned slightly east.

Experiments were also carried out with plants enclosed in a tall cardboard screen, which allowed light to fall only from above. An electric light was sometimes used to supplement the daylight intensity. Here again the leaves assumed a horizontal position. It is evident therefore the movement is not autonomous and is dependent on the direction of the incident light.

C. Observations in darkness.

Several experiments were done in an underground darkroom where the temperature and humidity were regulated at 24°C. and 85 % respectively. The movements were measured by means of a protractor

at intervals under an orange light.

The results were not uniform, as the plants showed nutations and nastic movements. Few leaves, however, did show more or less periodical movements, but they were not diaphototropic orientations in the sense that they were not regularly in the east-west direction and that the curvatures were not limited to the upper end of the petioles. They were, therefore, comparable to sleeping movement.

On account of the irregular and compound nature of these dark movements, they can not be easily analyzed. The same thing was observed by Denecke in his work. This characteristic seems to be quite general in plants having variation joints.

D. Observations under colored light.

In several experiments carried out in the greenhouse, plants were placed under two Senebier jars filled respectively with solutions of $CuSO_4$ and $K_2Cr_2O_7$. For three consecutive days the plants were exchanged daily from the bell jars and the movements of the leaves were carefully followed. The results of one such experiment are shown in table XXXII. (See next page.)

Except the first two leaves of plant 1, which were dying and therefore did not move, all the leaves showed good movements in the white light and under the blue filter, but none under the red filter. The intensities under the two bell jars might be different, but since this does not constitute an important factor in this movement (Vöchting 1888) it is justifiable to conclude that the red light is inactive diaphototropically

Table XXXII. Diaphototropic movements in colored light.

Date ----	April 30	May 1	May 2
Color ---	Blue	Red	Blue
Plant 1 - leaf 1	-	dropped off	
leaf 2	?	-	-
leaf 3	+	-	+
leaf 4	+	?	+
leaf 5	+	-	+
leaf 6	+	-	+
Color ---	White	Blue	Red
Plant 2 - leaf 1	+	+	?
leaf 2	+	+	-
leaf 3	+	+	-

III. Analysis of the movement.

A. The organ of perception.

To find out whether the lamina or the petiole is responsible for the perception of light, experiments were made as follows. Active leaves were chosen on several plants and their laminae or petioles (including the laminar joints) were shaded from light by means of black paper, rubber tubing, or India ink. Black paper was found to be the most satisfactory. The movements of these leaves were then observed for three consecutive days. The results as summarized in table XXXIII indicate that the laminae are the perceptive organs. Shading of the petioles does not stop the movement.

Table XXXIII. Diaphototropic movements of leaves with petioles or laminae shaded.

	Total no. of leaves obser.	No. of leaves showing diaph. movement.	No. of leaves not showing diaphoto. movement.
Controls --------	12	11	1
Petiole shaded --	11	10	1
Lamina shaded ---	9	0	9
Both petiole and lamina shaded ---	2	0	2

It ~~was~~ is known in some plants that the center of the leaf blade, i.e. the part just above the insertion of the petiole, is especially sensitive to light. This is, however, not true for Malva. In three experiments fifteen leaves were partially shaded either in the center or in the margin by means of black paper and India ink. All of them showed good movement. Therefore the whole blade is equally sensitive to light; as long as a part is exposed to light movement can be observed. The same results had been obtained by Denecke (1924).

B. The organ of reaction.

During the daily movement of the leaves the majority of the petioles remained straight and the angles they made with the stem were constant. This fact is clearly shown by figure 9. The curves represent the variations of the angles between the stem and the petioles of three leaves whose movements were given in figure 7.

In several other experiments the petioles were marked into sections and the movements were photographed and measured. Figure 10 shows the results of such an experiment using artificial light. The curve P_1 represents the rate of change of the angle between the lamina and

the petiole, P_2 the rate of change of curvature of the distal 5 mm. zone, P_3 that of the next 15 mm. zone, P_4 the next 20 mm. zone, and P_5 the last 20 mm. zone. It is evident from these curves that the upper 5 mm. section of the petiole which curved most is responsible for the movement. The next section curved very slightly and probably represents the phototropism of the petiole itself.

The active part of the petiole has been ~~regarded as~~ called the laminar joint (Pfeffer 1903). It is thicker than the rest of the petiole and has a peculiar anatomical structure in having only one central vascular bundle, while the petiole has six or seven strands arranged on the periphery. The change in the structure is gradual. At five to six millimeters below the lamina the petiolar bundles begin to fuse into a hollow ring. The central pith is then gradually eliminated until at about 2 mm. from the lamina it disappears, resulting in a single solid bundle. On entering the lamina the bundle first becomes horseshoe shaped and separates into seven strands which emerge into the main veins of the lamina. A joint having a central bundle surrounded by parenchyma is, teleologically speaking, very well adapted to the movement. Serial sections were made from this part of the petiole and are shown in figure 11. The structure of the variation joint has been studied by Vöchting (1888) and Pfeffer (1903).

IV. The mechanism of the movement.

A. Relation to growth.

The next phase of the problem that was undertaken was to find out whether the curvature movement is a result of growth or a variation in turgor. Experimental evidences indicate that this is not a growth movement, because:

(1) There is no relation between the grand period of growth and the activity of the diaphototropic movement. Very young leaves do not show this movement despite the rapid growth of their petioles. The nearly mature leaves show the most active movement. Mature leaves, which practically cease to grow, still show distinct movement for several days before they are shed (table XXXIV).

Table XXXIV. Movement after cessation of growth.

	Leaf number	Date of growth	cessation of movement	Days of movement after growth ceased.
plant ~~Part~~ 2. -----	1	3,19	3,24	5
	2	3,22	3,24	2
	3	3,30	4,1	2
	4	3,31	4,2	2
plant ~~Part~~ 3. -----	1	3,18	3,22	4
	2	3,19	3,22	3
	3	3,20	3,24	4
	4	3,31	4,1	2
	5	3,31	4,2	3
	6	3,30	4,2	3

(2) There is also no relation between the growing zones and the movement zones. We have seen that the curvature takes place only in the laminar joint, but the zone of rapid growth is always below the joint; in young leaves it is in the ~~basal~~ lower part and it moves upward as the leaves grow older but never reaches the joint (figure 12).

B. Extension and contraction of the moving laminar joint.

To verify that the movement is not due to growth, the variations in the lengths of the two sides and the median line of the joint

were measured during the movement. The measurements were made by means of a micrometer. The joint was about 4 mm. long, the accuracy of the measurements being within 0.5 micrometer divisions (1/30 mm.).

The results are shown in figure 13. While the length of the east side of the joint (upper curve) increased steadily from morning until 4 pm., the west side (lower curve) cohtracted proportionally, and consequently the joint curved toward the west. After sunset the phenomenon was reversed, both sides quickly regaining their original lengths, and the leaf faced east again. During the whole movement the length of the middle line of the joint remained unchanged. It is evident, therefore, that the curvature is not due to a differential growth, but to the reversible expansion and contraction of the two sides of the joint (Pfeffer 1903).

C. Osmotic relations in the joint.

To get a complete picture of the turgor relations between the two sides of the joint, it would be necessary to determine both the suction force and the osmotic pressure of the cells (Weidlich 1930). Both determinations, however, were found to be unsatisfactory and not accurate enough for this purpose. Therefore only the osmotic pressure at incipient plasmolysis (O_g) was measured. The plasmolytic method of Höfler (Brauner 1932) was used instead of the ordinary method of determining O_g because the cells were colorless and thus the plasmolytic condition could only be ascertained in its advanced stage (Ernest 1935). Each experiment consisted of several determinations of O_g at different times of the day. The same plant was used in one experiment and one joint was used for each determination.

The joint was sectioned and placed in sucrose solutions previously found to be isotonic or slightly hypertonic by the method of cell counting. A group of ten plasmolyzed cells in the cortex was drawn with the aid of a camera lucida. The area of the protoplast (A_p) and that exclosed by the cell wall (A_c) were measured with a planimeter. The value of O_g was then calculated by means of the expression:

$$O_g = M \frac{V_p}{V_c} = M\left(\frac{A_p}{A_c}\right)^{3/2}$$

These values were transformed into atmospheres according to the table given by Molz (1926). The results of two experiments are given below:

Table XXXV. Osmotic pressure at incipient plasmolysis.

Time	Sucrose M	A_p/A_c	V_p/V_c	MV_p/V_c	O_g atm.	Sucrose	A_p/A_c	V_p/V_c	MV_p/V_c	O_g atm.	Diff O_g atm.
		East side					West side				
Exper. 1. Greenhouse plant.											
9:45 A.	0.90	0.872	0.814	0.733	22.8	0.85	0.880	0.826	0.702	21.6	1.2
12:00 N.	0.90	0.860	0.798	0.718	22.2	0.85	0.845	0.777	0.661	20.0	2.2
3:00 P.	0.95	0.921	0.884	0.840	27.2	0.90	0.893	0.844	0.760	23.8	3.4
6:00 P.	0.95	0.862	0.801	0.761	23.8	0.95	0.900	0.854	0.811	26.0	-2.2
9:00 P.	0.85	0.898	0.851	0.724	22.5	0.85	0.880	0.826	0.702	21.6	0.9
11:15 P.	0.90	0.873	0.816	0.734	22.9	0.90	0.871	0.813	0.732	22.8	0.1
Exper. 2. Field plant.											
9:30 A.	0.95*	0.789	0.701	0.666	20.2	0.85	0.842	0.773	0.657	19.9	0.3
1:30 P.	0.80	0.840	0.770	0.616	18.3	0.80	0.740	0.637	0.509	14.6	3.7
6:00 P.	0.80	0.845	0.777	0.622	18.6	0.80	0.893	0.844	0.676	20.6	-2.0
10:30 P.	0.85	0.860	0.798	0.678	20.6	0.85	0.847	0.780	0.683	20.1	0.5

* Concentration too high, cells reached "Ball" stage of plasmolysis.

Since here we are dealing with only the undifferentiated, thin-

walled cortical cells in the close vicinity of vascular tissue, it seems reasonable to consider that the suction force is the same in the two sides and that the O_g is the primary factor determining the turgor pressure of the cell. If this is true, the mechanism of the movement becomes explicable. It can be seen from table XXXV that during the day the O_g of the cells in the east side of the joint was always higher than that in the west side, and caused the joint to curve toward the west. At sunset the difference in O_g was reversed and so was the returning movement. Finally at night the O_g's were equal and the movement stopped.

Perhaps some idea as to the nature of the osmotic changes can be obtained if the values of O_g at different times of the day are compared. At midnight the O_g of the two sides ~~is~~ was the same. In the morning, while the O_g in the east side remained constant, it decreased in the west side, indicating a loss in osmotically active substance. In the afternoon the O_g increased in both sides, the increase being more at first in the east side and then in the west side. In the night the O_g dropped off again.

V. Conclusions and discussion.

In the first part of the investigation it has been shown that light is the causative factor for the diaphototropic movement of the leaves of Malva. These results confirm the hypothesis proposed by Franck (1870) and experimentally established by Darwin (1881) and Vöchting (1888). By the use of a clinostat these authors proved that geotropism, epinasty, etc. have nothing to do with the movement and light alone is important. The present investigation further shows the direction of the light that is essential for the orientation.

For the perception of light the leaf blade is responsible. In this respect Malva is comparable with such plants as Tropaeolum (Denecke 1924) and unlike Fuchsia and Phaseolus (Pfeffer 1903). No attempt has been made to study the mechanism of perception as the knowledge in this field is far too scanty. The only theories known are those of Haberlandt (1905) and Wager (1909). Since both of them have been found to be unsatisfactory they will not be discussed here.

The mechanism of the reaction is of particular interest to us. Both Vöchting and the present paper showed that the laminar joint is alone concerned in the movement. The bending of the joint during movement is not due to growth because, while one side extends the other side contracts. It is due to a reversible change in turgor on the two sides as shown by osmotic pressure measurements. The change in osmotic pressure may be due to either a change in permeability of the protoplasm or the formation and precipitation of the osmotically active substances (e.g. starch $\rightleftharpoons$ sugar). The permeability change is probably the cause of the quick movements, e.g. in Mimosa (Pfeffer 1903) and in Sparmannia (Bünning 1930); the anatonosis and precipitation of the osmotic substances are probably mainly responsible for the slow movement, such as we have here in Malva.

Recent work on the mechanism of ~~longer~~ turgor movements has been chiefly done on Phaseolus. The primary leaves of this plant exhibit both photonastic and sleeping movements. Unlike Malva the laminar joints of Phaseolus are found to be dorsoventrally dissimilar (Weidlich 1930, Brauner 1932). The osmotic changes during the nyctinastic movement have been studied by Weidlich (1930) who found that the movement

is due to the alternate increases and decreases of osmotically active substances in the upper and lower sides of the joint. These results are therefore comparable to that those we have found in Malva. Later work by Brauner (1932) and Bünning (1935), however, inclined them to explain such changes on the basis of changes in the water permeability. Thus Brauner believed that the photonastic movement is due to the increase in permeability of the upper side of the joint and Bünning considers that the nyctinastic movement is caused by the differences in the permeability on the two sides of the joint. Since no conclusive data have ever been presented, the permeability hypothesis can not be regarded as established. No mention has been made in this discussion about the elasticity of the cell wall, although it has been suggested that the change in wall elasticity may play a role in turgor movements (Zollikofer 1935).

As to how the stimulus is conducted from the lamina that perceives the light to the joint which gives the reaction, nothing is as yet known. That the conduction is through the veins has been shown by Denecke (1926). In our experiments with artificial light we found the reaction time was long (more than half an hour). This suggests that the rate of conduction is rather low. In this respect it differs from the seismonastic turgor movements of Mimosa or Sparmannia, the reaction time of which is a matter of a few seconds. Perhaps the conduction here is of a chemical nature comparable to the slow conduction of Mimosa (Fitting 1930) or in floral stalks (auxin, Zollikofer 1935).

VI. Summary.

(1) The daily diaphototropic orientation of the leaves of Malva neglecta was studied with the aid of a motion picture camera. The leaves were found to follow closely the course of the sun, with their faces always perpendicular to the incident rays. After sunset they turned back to their original position (facing east).

(2) No such orientations of leaves have been found in evenly diffused light and in continuous darkness. The direction of the incident light is therefore important.

(3) Plants kept under red light showed no diaphototropic movement, but they showed such a movement under blue light.

(4) Leaf-laminae were found to be the organs of perception for ~~the~~ light.

(5) Diaphototropic movement (curvature) has been found to be limited to the upper 4 or 5 mm. of the petiole. This part (laminar joint) possesses different properties (structural) than the rest of the petiole. It has a central bundle instead of several peripheral ones.

(6) The curvature is not due to growth because:

a. no relation exists between grand period of growth and movement activity.

b. no relation exists between the growing zones and the movable part (laminar joint).

(7) The curvature has been found to be the result of extension and contraction of the two sides of the joint. These changes in length are completely reversible and therefore do not

result in growth.

(8) By measuring the osmotic pressure (O_g) of the cells at different times of the day, it can be shown that the movement is due to a difference in turgor between the two sides of the joint.

(9) The results were discussed in connection with the literature on turgor movements.

V

Nyctinastic Movement of Leaves of Carica papaya

I. Introduction.

Nyctinastic or sleeping movement of the leaves and flowers of plants constitutes one of the oldest problems in botany. It was first observed in some plant by Pliny (23 - 79 A.D.) and then by Albertus Magnus (1200 - 1280). The common occurrence of these movements in plants was shown by Linnaeus. Subsequent researches were mainly concerned with the nature of the mechanism of the movement. A.P. de Candolle was able at first to shift the normal course of flower movement by the use of artificial light, which lead him to believe the movement was paratonic. Later, however, he failed to obtain the same results in other plants and accordingly changed his view so that he ~~now~~ then believed the movement was autonomic in nature. The later view of de Candolle was adopted by Dutrochet, Sachs, and Hofmeister. Sachs said "Die periodische Bewegungen an sich ist unabhangig von dem Wechsel der Beleuchtung, aber die periodische Bewegung in dem Zeitmaß wie sie unter gewöhnlichen Verhältnissen auftritt wird durch den Lichtreiz bestimmt" (Stoppel 1910). The conception of sleeping movement prevailed for several years until Pfeffer (1875), who did the most extensive work on the subject, concluded that the movement was a direct after-effect of the normal alternation of external conditions and that autonomic periodicity, if it existed, was of secondary importance. Later, partially through the influence of Semon, Pfeffer (1907 - 1915) reinvestigated the question and modified his view to one that was somewhat similar to that of Sachs.

Pfeffer considered that, while in some plants (flower of Tulipa) the movement was caused by external variations, in others (Phaseolus) it was a combination of an autonomic periodicity and a paratonic reaction to the daily variation of environment. After Pfeffer the question was continued by Stoppel (1910 - 1933), Brauner (1926), and Cremer (1923) who attached more importance to the external conditions and Kleinhoonte (1929), Schmitz (1934), and Bünning (1932) who believed in the existence of a "tagesautonomer" periodicity. The question up to the present is still unsolved.

Parallel with the study on the nature or cause of the periodicity numerous investigations were done on the mechanism of the movement. The first important work was done by Pfeffer who showed by microscopical measurement that in "jointless" leaves the movement was due to the unequal growth of the two sides of the petiole, while in the leaves with movable joints the movement was a result of turgor change. Later work extended these results to a number of different plants. Thus in most Oxalidaceae and Leguminosae, e.g. Phaseolus, the movement was definitely shown to be due to the difference in turgor on the two sides of the joint (Bünning 1934, Weidlich 1930, and Brauner 1932). In Coleus the movement was found to be a result of differential growth of the petiole (~~Selimitz~~ Schmitz 1934). And finally in some tropical plants the movement could be resolved into two components, one due to turgor and the other to growth (Metzner 1934).

Various teleological suggestions were given as to the possible biological significance of the sleeping movements. At first they were compared to the fatigue reaction in animals, a contention removed by

Jost. Protection from cold or frost by reduction of the exposed surface was suggested by Darwin (1881), and a reduction of transpiration was suggested by Stahl (Pfeffer 1903). Speculations of this nature were, however, outside the scope of the present investigation.

Sleeping movements are known in a large number of plants belonging to various families, e.g. Amarantaceae, Balsaminaceae, Solanaceae, Leguminosae, Oxalidaceae, Marantaceae, etc (Kostychew and Went 1931). In the present investigation a species of Caricaceae (Carica papaya) was used. The purpose was to find out the nature and the mechanism of the sleeping movement in this form and compare them with the results obtained with other plants in the literature.

II. Material and method.

Papaya plants (Carica papaya) of a pure strain, kindly supplied by Dr. Marshall of La Jolla, were used throughout. The seeds were germinated in sand, transplanted into flower pots and fed daily with nutrient solution of the following composition:

$Ca(NO_3)_2$	0.005 M
$MgSO_4$	0.005 M
KH_2PO_4	0.003 M
$Fe_2(C_4H_4O_6).H_2O$	trace
HCl	2.8×10^{-4} M
H_2O	1000 c.c.

Experiments were made with plants when they were about six to ten inches high.

In the first few experiments the movement of the leaves was

followed by measuring the angle between the leaf lamina and the petiole with a protractor. Later a self-recording device of Pfeffer (1915) and Kleinhoonte (1932), with suitable modifications, was used. The apparatus consisted of an electic clock driving a rotating drum ~~mounted on an electric clock~~ which made approximately one revolution in one week. A glass capillary filled with glycerine ink was used as a stylus. It was fastened to the mid-rib of the leaf with silk thread over a pulley. This method has the advantages over the lever stylus and ~~method or the~~ smoked paper method in that it goes vertically up and down, instead of describing an arc, and that several records can be made simultaneously on the same drum by using different colors of ink.

To check the results obtained on the kymograph the movement of two leaves were ~~one leaf was~~ recorded by means of a cinematographic camera, which automatically made one exposure every fifteen minutes. The results are plotted in the figure. (Fig 15)

III. The normal course of the movement.

The course of the diurnal movement of leaves under normal conditions in the green house was recorded. The results are given in figure14 in which the peaks of the curve represent the day position and the valleys the night position of the leaves. (Plate VA and VB).

A cinematographic record has also been made. It is found in figure 15.

Several facts were observed in these figures: (1) The period of the movement was constantly 24 hours in all experiments. The leaves gradually rose from the night position at or before midnight and attained the day position in the morning. In the evening they dropped

rather quickly and reached the night position in less than three hours. The rising of the leaves before midnight indicated that the movement was not a result of the variation in illumination. (2) The amplitude of movement was, on the other hand, variable. It was different for leaves of different ages (Chapter VI) and under different external conditions. Figures 14, experiment 1 serves to illustrate this point. The movement had a smaller amplitude in rainy and cloudy days. A similar decrease was observed in the winter. In extreme cases the plant ceased to move at all. The decreased activity was probably due to a lower rate of growth (Chapter VI). (3) In some leaves a slight drooping reaction was observed at noon (figure 14, leaf 11 - 22). Similar phenomena had been observed in other plants, e.g. Oxalis, Mimosa, especially in the tropics (Ewart 1894). Such movements commonly called "midday sleep" are probably due to the high light intensity at noon.

IV. Movement under constant illumination.

To find out whether the movement ~~is~~ was induced by the normal alternation of day and night, observations were made on plants in a constant light and constant darkness.

A. Leaves grown in the green house.

For the light experiments plants were brought from the green house to an underground room and constantly illuminated with 200 watt incandescent lights. The room temperature was fairly constant at 23 - 25°C. Several experiments were made in which the movement was either recorded automatically or followed with a protractor. The results (figures 16 and 17) show that: (1) In general the leaves remained active several days after being brought under constant light.

Individual leaves differed in this respect (Kleinhoonte 1932). Less active leaves usually showed movement for three to six days (experiment 3), but the most active leaf (experiment 2 not shown) continued to move regularly for more than twenty days. The final cessation of movement in this case was due to the stoppage of the growth. It is difficult to say whether the movement should be considered as autonomous or as an after-effect (Nachwirkung) of the environment (Pfeffer 1907). (2) The period of the movement was approximately twenty four hours. In some the period was slightly longer. This resulted in the day and night positions being shifted after a period of time so that they did not coincide with the actual day and night. (Figure 16 shows this.) (3) The amplitude of the movement was always smaller than that under normal conditions, although in the most active leaves the amplitude was as large as the normal in the first two or three days under constant illumination (figure 16). The decrease in amplitude was due to a decreased tendency of the leaves to sleep. They then remained more or less in the horizontal position. (4) When the leaves were brought back to the green house they quickly resumed their active movement. The amplitude increased due to the more pronounced sleeping reactions (figure 16).

B. Leaves grown in constant illumination.

Observations were also made on leaves that developed from buds under constant light (figure 18) and no distinct periodical movement could be noticed. When these leaves were brought into the green house the sleeping action at once appeared. This indicates that the diurnal alternation of external conditions, e.g. light, temperature, etc., acts

as a stimulus to the leaf movement, although it is perhaps not the sole cause of the periodicity (section VI).

It is well to mention that papaya leaves are also found to be diaphototropically sensitive. The leaves that had been grown in constant illumination, or had been there a sufficient length of time, arranged themselves perpendicularly to the indident radiation (Chapter IV). This phenomenon will not be included in the present investigation.

V. Movement in darkness.

Plants which showed active movement in the green house were brought into the darkroom, the temperature of which was regulated at 24°C. and the humidity at 80 %. They were kept in complete darkness, except at the time of watering when they received orange light for two or three minutes (marked by arrows in figure 19). Since the leaves when brought into darkness showed strong epinastic curvature at the base of the petiole, the lower part of the petiole was fastened to a glass rod. This procedure did not interfere with the normal sleeping movement which was limited to the upper part of the petiole.

The results (figure 19, experiments 12 and 13) show: (1) That the leaves retained their activity in the darkness for six to seven days. An examination of these leaves showed that, after the movement stopped, the leaves were etiolated and frequently abscissed. The loss of activity was therefore due to the death of the leaves. Such movements are perhaps not an after-effect of the environment. (2) The period of the dark movement was in general shorter than the normal. It was around twenty one hours instead of twenty four (figure 19).

Similar results were found in Phaseolus by Bünning (1932) who suggested the rise in temperature as the cause of the accelerated movement. (3) The amplitude of the movement was comparable to the normal at first but decreased somewhat after several days. (4) The small exposure to orange light had no influence on the movement. It can be seen from the curves that these light periods had no relation with the subsequent rising or lowering movement of the leaves. And in one experiment two leaves which were left in complete darkness for three days showed no decrease in activity (figure 19, experiment 12). It seems unlikely that the movement was stimulated by the small exposure to light (Bünning). (5) Apparently no relation existed between the movement and the time at which the plants were brought into the darkness, i.e. the rising or sinking reactions were not shifted if the plants were darkened at noon or at night (experiment 3 not shown). In this respect the movement of papaya differed from that of the primary leaves of Canavalia. It was found that in the latter case the course of the movement was shifted according to the time at which the plants were brought into darkness (Kleinhoonte 1932).

VI. Experiments on the clinostat.

In several experiments the plants were mounted on a clinostat which turned slowly around a horizontal axis. Under this condition the leaves did not show the normal sleeping movement but gave strong epinastic curvature at the base of the petioles. Similar phenomena have been observed in Phaseolus, Canavalia (Kostychew and Went 1931) and Coleus (Schmitz 1934). These curvatures are not related to the normal nyctinastic movements and therefore will not be discussed in detail.

VII. Discussion on the nature of the movement.

The question, whether the sleeping movement of papaya is autonomous or aitiogenic can not be easily answered. A dilemma has existed between these two terms since the time of de Candolle. No clarification has as yet been found after ~~two~~ one centuries of research. As Stoppel had plainly remarked, we are at present not much further in our knowledge of the nature of this movement than we were in the year 1759 (Bünning 1932).

According to Pfeffer, autonomic movement is that "bei voller Konstanz der Aussenverhältnisse durch ein selbstregulatorisches ~~selbstregulatorisches~~ Walten veranlasst und dirigiert werden" (Pfeffer 1907 and page 401.). As long as all the external factors and the internal regulatory mechanism are not completely known, it seems to the writer impossible to assign to any movement as autonomy, according to this definition. Because, no matter how many factors are known and be brought under control, there will always be the possibility of some uncontrollable factor, e.g. atmospheric conditions (electricity) (Stoppel 1920, 1933) or an imaginary factor ~~designable by~~ X (~~Brauner~~ Brouwer see 1926).

Another thing that adds confusion to the problem is the after-effect (Nachwirkung) (Pfeffer 1903) of the environment. According to this view the movements of leaves under constant conditions is due to the cumulative effect of the normal daily alteration between light and dark to which the plants are subjected previously. In some plants which only show movement for one or two days after being brought into constant condition, the after-effect can perhaps be defined. Here it seems difficult to define a limit to this after-effect, i.e. one may

regard the movements that last for one or two days as due to the after-effect or may as well regard movements of seven or eight days (which is already the whole lifetime of some leaves) as due to the after-effect. The only criterion that can be used then is the inheratibility of such movement, but experiments of this nature are not always easily done with any plant and the results are not always convincing (Semon 1908). Another objection to this after-effect seems to be reasonable. Pfeffer compared the building up of the response to the oscillation of a pendulum induced by a series of rhythmically repeated impulses. If this is true we would expect the movement to build itself up gradually when an inactive plant is brought to the rhythmic alternation of illumination. This, however, is not the case as we have shown (section IV) for as soon as the inactive plant is moved to the green house the movement starts immediately and has the same amplitude as the normal movement.

Bünning in his work on Phaseolus (1932) has given another criterion or interpretation of autonomous movement. He considers the movement autonomous when "Die Periodizität wird nicht durch einen äussenen Faktor mit tagesperiodischer Schwankung verursacht". In this way he showed the autonomous nature of the movements of the primary leaves of Phaseolus because the period of the movement was not always twenty four hours in the darkroom at a slightly higher temperature, and in fact could be varied with the temperature. Since we have shown that papaya leaves have a period of less than twenty-four hours in the darkroom at 24°C. (section V), we can on the same grounds regard this movement as autonomic in nature, whereas according to Pfeffer the movement would perhaps be considered as aitiogenic

and the movements under constant illumination or darkness as the simple expressions of the after-effect.

Three recent works on autonomic movement of leaves were found in the literature. Bünning (1932) proved the autonomic nature of nyctinastic movement of the primary leaves of Phaseolus and (1932) also showed that the periodicity was heritable. Kleinhoonte (1932) found the same conditions in the leaf movements of Canavalia. Finally Schmitz (1934), with similar methods, found that while the twenty four hour periodicity of the movement of the leaves of Coleus was autonomic, the time of the rising and drooping of the leaves depended upon an unknown "Steuerungsfaktor", a contention resembling that of Sachs. Schmitz's work is of special interest to us because in Coleus, just as in papaya, the movement is due to differential growth (Chapter VI).

VIII. Summary.

(1) A short historical sketch of the study on the nyctinastic movement is given.

(2) The method of automatic recording of leaf movements is described.

(3) The normal nyctinastic movement of papaya leaves has a period of about twenty four hours. The leaves rise at about midnight and reach the horizontal position in the morning. They dropp in the evening and become vertical at night.

(4) The amplitude of the movement depends on external conditions. Some leaves show midday sleep at high noon.

(5) In constant light the movement persists for three to six or even to twenty days. The period of the movement remains twenty four

hours but the amplitude is always smaller.

(6) Leaves grown in constant illumination do not show periodic movement, but as soon as they are brought into the normal daily alternation of illumination the movement commences.

(7) The movement continues in the dark. The period is approximately twenty one instead of twenty four hours, perhaps due to the higher temperature of the darkroom.

(8) The question of autonomic or aitiogenic periodicity of leaf movement is discussed. The conclusion is reached that the nyctinastic movement of Papaya ~~neglecta~~ leaves is of an autonomic nature.

VI

Nyctinastic Movement of Leaves of Carica papaya

Mechanism of Movement

I. Relation of growth to movement.

It has been said in the foregoing chapter that nyctinastic movements in plants are either brought about by the turgor changes in, or the unequal growth of, the two sides of the petiole (Pfeffer 1903). To find out which of these mechanisms is responsible for the nyctinastic movement in papaya, experiments have been made along three lines:

(1) By studying the movement of leaves of different ages, i.e. leaves of different growth rates.

(2) By comparing the location of the movement with the growing zones of the petiole.

(3) By direct measurement of the changes in length of the two sides (abaxial and adaxial) of the petiole during the movement.

A. Movement of leaves of different ages.

Observations were made on the movements of all the leaves of a single plant. The leaves were numbered according to the order of their emergence from the buds. The smallest number therefore represented the oldest leaf and the largest number the youngest leaf. The age difference between two consecutive leaves was about a week. The results are plotted in figure 20 and for convenience only the alternative leaves are given. The figures show clearly that the

oldest leaf (No. 7) was inactive, the youngest leaf was fairly active, and the leaves intermediate between these ages were the most active in nyctinastic movement.

In figure 21 the growth curves of the petioles of two leaves ~~is~~ are given. The curves were not extrapolated to the origin because the definite time of the emergence of the leaves could not be easily ascertained. It is seen from the curves that the petioles shows a distinct grand period of growth from approximately the time they emerged until they were five or six weeks old. After that they ceased to grow and somewhat later abscissed.

A comparison of figure 20 and figure 21 shows that there is a close parallelism between the activity of movment and the activity of growth of the leaves. The rapidly growing leaves showed the most active movement, while the mature and non-growing leaves had ~~stopped~~ ceased to move. This fact gives the first evidence that the movement is caused by growth (compare Malva, Chapter IV).

B. Localization of movement in the growing zones.

It has been observed that the nyctinastic movement, i.e. curvature, is confined to the upper third, or fourth, of the petiole (Chapter V, plate V). It remains to find out whether this bears any relation to the growing zone of the petiole. The petioles were marked into one or two millimeter sections with India ink and the growth of these sections was measured weekly with a pair of calipers. The results are shown in figure 22. It is seen that at the very young stage the whole length of the petiole grew. Later two growing zones appeared and gradually moved toward the two ends of the petiole, the

one at the laminar end being more active than the one at the stem end. Careful observation revealed that the movement zone coincides with the laminar growing zone of the petiole. This gives a second evidence for the growth mechanism of this movement.

The growing zone at the stem end of the petiole is not concerned in the nyctinastic movement as the lower part of the petiole remains stationary during the diurnal movement. When the plants are brought into darkness, or on a horizontal clinostat, this lower zone is stimulated and gives rise to a pronounced epinastic curvature.

C. Differential growth of the two sides of the petiole.

Final proof of the growth nature of the nyctinastic movement was obtained by direct measurement of the lengths of the upper (adaxial) and the lower (abaxial) sides of the petiole during movement. For this purpose the distal third of the petiole was marked into sections on both sides and measurements were made with the aid of a horizontal microscope at different times of the day. The results are given in table XXXVI and plotted in figure 23.

It is seen (figure 23, curves A and B) that the upper (adaxial) side of the petiole grew only during the later part of the day (from noon to midnight) and did not grow at all in the earlier half of the day (from midnight until noon). The reverse was true of the lower (abaxial) side, but to a less marked degree, i.e. the growth slowed down in the afternoon and evening, but never completely stopped.

If the differences in lengths of the two sides of the petiole at different times of the day is plotted against time, a curve is obtained (figure 23, curve C) which is almost exactly superimposable

Table XXXVI. Differential growth of the upper and lower sides of petiole.

Time		Lengths in mm. upper side	lower side	upper minus lower
4/1	½	24.11	22.79	1.33
	12	24.08	24.01	0.07
	18	24.45	24.25	0.20
4/2	0	24.71	24.35	0.35
	9½	24.72	24.73	-0.01
	14	24.99	24.88	0.11
	17½	25.39	25.02	0.37
	20¾	26.12	25.07	1.05
	23¾	26.32	25.31	1.01
4/3	10	26.37	26.34	0.03
	12	26.46	26.44	0.02
	15	26.51	26.55	-0.05
	18	27.59	26.67	0.92
	21	28.38	26.97	1.40
	23½	28.58	27.39	1.18

on the movement curve (figure 23, curve D) for the same leaf. The movement curve was obtained ~~from~~ on the ~~automatic reading drum~~ kymograph (see Chapter V).

The quantitative agreement between the curves of growth and movement gives a definite proof that the movement is due to the differential growth of the upper and lower sides of the petiole.

II. Auxin in relation to growth and movement.

It has been established that auxin is the primary factor for the growth in length of plants (Went 1928). The unequal growth in phototropic and geotropic curvatures of plant parts is due to an unequal distribution or destruction of auxin (Went 1928, Overbeek 1932). The next problem here was then to find the relation of auxin to the differential growth of the petiole of papaya. Experiments were conducted to find out:

(1) where auxin is produced, and

(2) how is it distributed.

A. Effect of debladding on growth and movement.

Three experiments were done with (1) normal leaves, (2) leaves from which the blades were removed excepting the midrib (debladed leaves), and (3) debladed leaves with a small amount of auxin paste applied to the point of insertion of the petiole at frequent intervals. The auxin concentration was 1 part in 1000 parts of lanolum. The movements of these leaves were registered on the kymograph. The growth of the two sides of the petioles was measured at twelve hour intervals with the aid of a horizontal microscope. The movment curves of the debladed leaves and the debladed-auxin leaves are given in figure 24 and 25 respectively. The growth measurements of all the three kinds of leaves are tabulated in table XXXVII and plotted in figure 26.

The results show that: first, the debladed leaves have lost their activity for both growth and movement, indicating that auxin is only produced in the blade. Second, the debladed-auxin leaves cease to move but still continue to grow. The growth, however, is equal in the two

follows 77

Table XXXVII. Growth of petioles: normal, debladed and debladed plus auxin.

Time	% of ~~growth~~ increase in length					
	normal		debladed		debladed plus auxin	
	upper side	lower side	upper side	lower side	upper side	lower side
0	0	0	0	0	0	0
12	0.19	3.10	-0.6	4.4	5.7	6.9
0	3.95	5.93	1.8	3.2	13.9	13.8
12	3.91	8.35	0.2	3.5	16.3	18.2
0	8.43	9.85	0.8	4.4	16.9*	21.0*
12	8.12	13.70	1.9	4.1	20.5	25.5
0	13.05	15.00	2.0	5.1	20.6	24.3
12	12.40	17.8				

* green house fumigated.

sides of the petiole, consequently no movement being observed. The capacity of growth, or sensitivity to auxin, of the two sides of the petiole is, therefore, the same. The normal movement must be due to a differential supply of auxin from the leaf blade. The whole mechanism of the movement must then be sought for in the blade.

B. Anatomical structure of the leaf.

In order to understand the differential supply of auxin from the blade to the two sides of the petiole, it is necessary to know the structural connection between these two parts. Microscopic sections were therefore made of the principal veins and also of the junctions between the leaf blade and the petiole (figure 28).

The gross appearance of the papaya leaf is shown in figure 27. The first few leaves produced by the young plant are three-lobed, later leaves are five-lobed and in mature plants the blades are generally seven-lobed. The principal veins from these lobes come together at one point and enter into the petiole at that point.

Since it is known that the conduction of auxin is chiefly through the phloem, special attention ~~was~~ is therefore paid to this tissue. In the principal vein (figure 28, A, B) the phloem consists of a number of strands surrounding the xylem. At the junction the xylem ~~shrunk~~ strands from all the veins and fused together (figure 28C) while at least part of the phloem strands (those on the ventral side of the veins) do not fuse and enter individually into the petiole (figure 28D, E, F). It is important to note here that, while most of the phloem strands from the central tip lobe (figure 27A) go to the lower (abaxial) side of the petiole, those from the basal lobes (figure 27D) enter

the upper (adaxial) side of the petiole and those from the side lobes (figure 27B) go to the lateral sides. A natural deduction from the study of the structure is that the differential supply of auxin, probably due to the different production or distribution in the different regions of the leaf blade, determines the movement. This deduction leads to the following experiments.

C. Movement induced by the application of auxin.

① Experiments in constant light.

To eliminate the normal nyctinastic movement the plants were placed under constant light (3 x 100 watts) for two weeks. The leaves that no longer showed nyctinastic movement were used for the experiment. Auxin pastes made of 1 part of pure heteroauxin in 500 or 1000 parts of lanolin was placed on the four basal lobes of the leaves (figure 27CC,DD) and covered with black paper to protect it from being destroyed by light (Overbeek 1935). The movement of the leaves was registered on a kymograph. The results of two experiments are given in figure 29, in which the arrows indicate the time of application of the auxin, of concentration shown in the brackets. An arrow above the curve means auxin applied on the upper (dorsal) side of the leaf, and an arrow below the curve means auxin put on the lower (ventral) side. Of the six leaves tested only one failed to give a reaction. The leaves treated only on the upper side with the 1/1000 auxin paste bent down slightly for about eight hours after the application. Then, in general, the leaves bent back to their normal horizontal position. Those leaves treated on both sides with the 1/500 auxin paste bent down much more and did not go back to the original position.

The curvature induced by auxin (especially the higher concentrations) appeared a little lower down the petiole (upper third) than that found in the nyctinastic movement normally. The explanation is perhaps that the higher concentration of auxin goes farther down the petiole than the much smaller amount of auxin normally found in the leaf.

It must be emphasized that although the auxin curvatures are slightly lower down the petioles , they are by no means comparable to the epinastic curvatures found at the very base of the petioles (section IB). Therefore they can not be considered as epinastic curvatures and are perhaps different from the auxin-induced epinasty in other plants (Hitchcock and Zimmerman 1935).

② Experiment in the greenhouse

Similar experiments were performed in the green house, the results of which are shown in figure 30 and 31.

It is seen that the application of auxin (1/1000) to the basal lobes (figure 27CC,DD) was very effective in causing the leaves to bend down to the sleeping positions. The reaction occurred within two hours after application of auxin.

The reverse reaction, i.e. bending upward of the leaves to the day position was more difficult to be induced by the application of auxin to the midrib (figure 27A). The 1/1000 auxin paste was ineffective, a stronger paste 1/500 was necessary. The rising reaction started either immediately or several hours after the treatment.

The leaf can therefore be either "put to sleep" or "awakened" at any time of the day by the artificial application of auxin to the different regions of the blade.

D. Extraction of auxin from leaves.

Direct proof of the differential supply of auxin from the blade must, of course, rest on the actual measurements of the auxin ~~exhausted~~ extracted from the leaf. A large number of experiments have been made using both the agar-diffusion method (Went 1928) and the $CHCl_3$ extraction method (Thimann 1934). So far the experiments have not been successful. The amount of auxin extractable from the leaves was always so small that quantitative measurement with both the normal and the deseeded Avena tests (Skoog 1937) has not been accomplished. The reason for this apparent failure is probably the high destructive activity of the cut surface of the papaya leaves. Both oxidases and peroxidases are present in comparatively large quantities as shown by the α-naphthol and benzidine-H_2O_2 tests (Overbeek 1935). It is hoped that further improvement of technique will make quantitative extraction possible. Experiments are now in progress relating to this phase of the subject.

III. Conclusions and discussion.

From the results of the above experiments a rough picture of the movement can be obtained. At midnight and in the morning <u>all</u> the auxin comes from the tip (central) lobe of the blade to the lower (abaxial) side of the petiole, so that the lower side grows while the upper side does not. The leaf, therefore, rises to the horizontal day position. In the late afternoon and the evening <u>more</u> of the auxin comes from the basal lobes of the blade to the upper (adaxial) side of the petiole, so that the upper side grows faster than the lower side. The leaf, therefore, bends down to the vertical night position.

Whether the different amount of auxin in the apical and basal lobes of the leaf blades is due to different production or distribution, can not be said definitely at present since direct measurement has not been accomplished. Evidence, however, is in favor of the latter possibility. Skoog (1937) and Avery (1935) found that the leaves produce auxin only in the light and use up their auxin in darkness in the course of a few days. Since papaya leaves exhibit the same movement in darkness, it is evidently not concerned with the production of auxin, but probably with the distribution of the accumulated auxin. The explanation of leaf movements from the hormonic point of view was first suggested by N. Ball (1923). Later experiments have been chiefly concerned with induced movement by the artificial application of auxin. Thus auxin has been found to induce epinasty and hyponasty in leaves of tobacco (Avery 1935), Coleus (Laibach and Fischnich 1936, Fischnich 1935), tomatoes (Hitchcock and Zimmerman 1935), etc.

Of special interest are two recent papers on floral movements. Böhner (1932) found that the opening of the flowers of Tulipa is due to an increase in the wall extensibility of the cells on the upper side of the sepals, and the closing action was due to the reverse process. The results were interpreted by Guttenberg (1933, 1935), as perhaps caused by a differential supply of auxin. Zollikofer found the same changes of wall extensibility in the curved inflorescence stalks of some plant (Tussilago & papaver). She was able to demonstrate further that these changes were primarily due to the difference in the amount of auxin. As far as the mechanism alone is concerned, these

floral movements are, therefore, very similar to the leaf movements of papaya.

IV. Summary.

(1) A close relation is shown to exist between the activity of nyctinastic movement and the age, i.e. the growth rate of the leaves.

(2) The curvature of the nyctinastic movement is localized in the upper growth zone of the petiole.

(3) Direct measurement shows that the movement is due to the unequal growth of the two sides of the petiole. The upper (adaxial) side grows only in the latter half of the day (noon to midnight) and does not grow in the earlier half (midnight to noon). The lower (abaxial) side grows continuously, although it grows more in the morning than in the afternoon. The course of movement agrees quantitatively with the difference in growth of the two sides. Therefore the unequal growth must be due to the differential supply of auxin from the leaf blade.

(4) The sensitivity to auxin of the two sides of the petiole was found to be the same, ~~which further supports the idea of a differential supply of auxin being an important factor.~~

(5) Nyctinastic movement of leaves can be induced by the external application of auxin. The leaves droop when auxin is applied to the basal lobes of the blade, and rise when the auxin is applied to the tip (central) lobe.

(6) Microscopical studies reveal that the main vein of the tip (central) lobe goes into the lower (abaxial) side of the petiole, while the main veins of the basal lobes enter into the upper

(adaxial) side.

(7) The conclusion is reached: that a differential auxin supply from different regions of the leaf blade causes a differential growth of the upper and lower sides of the petiole, which is responsible for the nyctinastic movement.

(8) The results are discussed in relation to the current literature.

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FIG. 1 DISTRIBUTION OF CELL SIZE

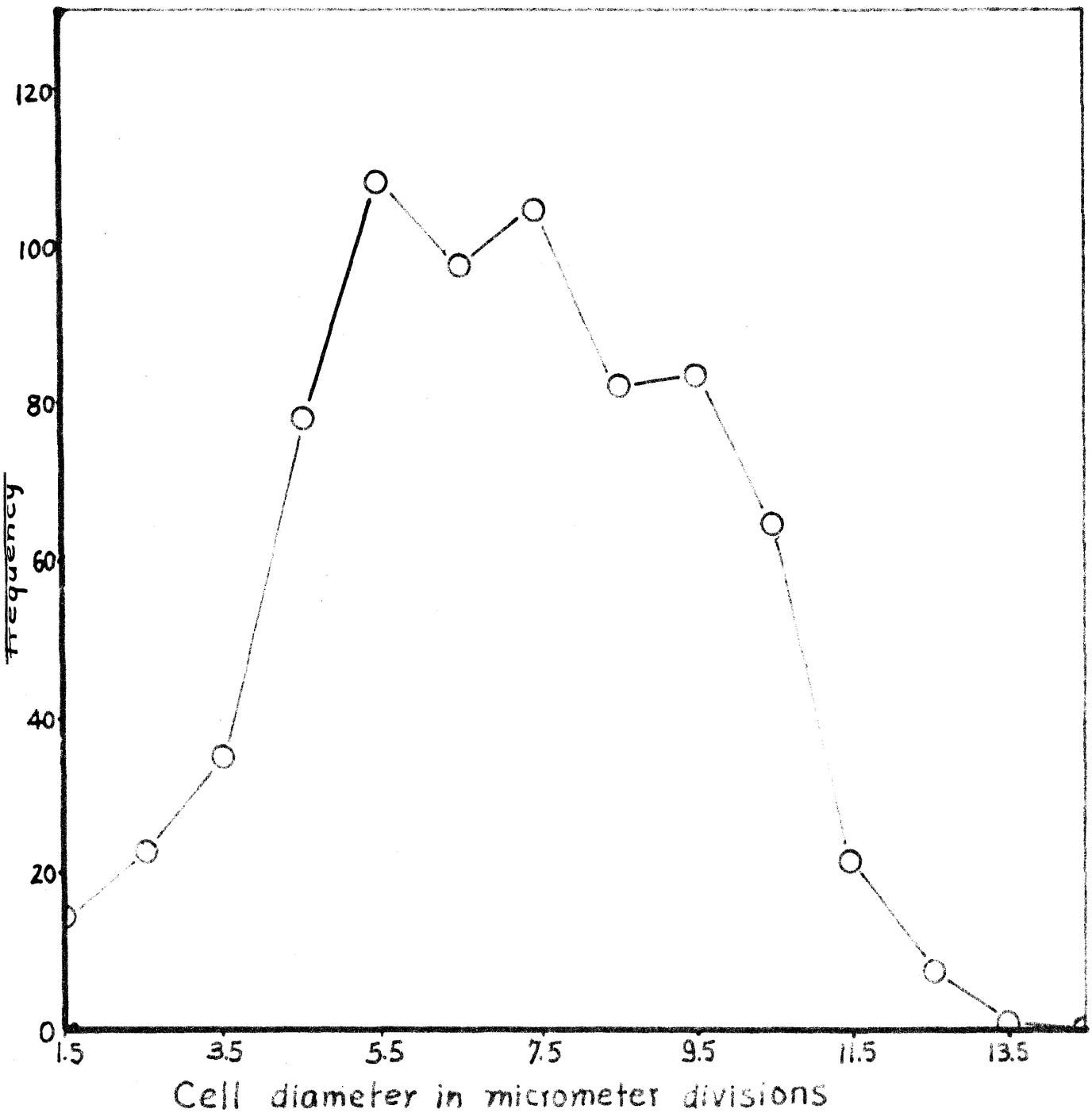


FIG. 2 CELL SIZE IN A PURE CLONE (Aisa)

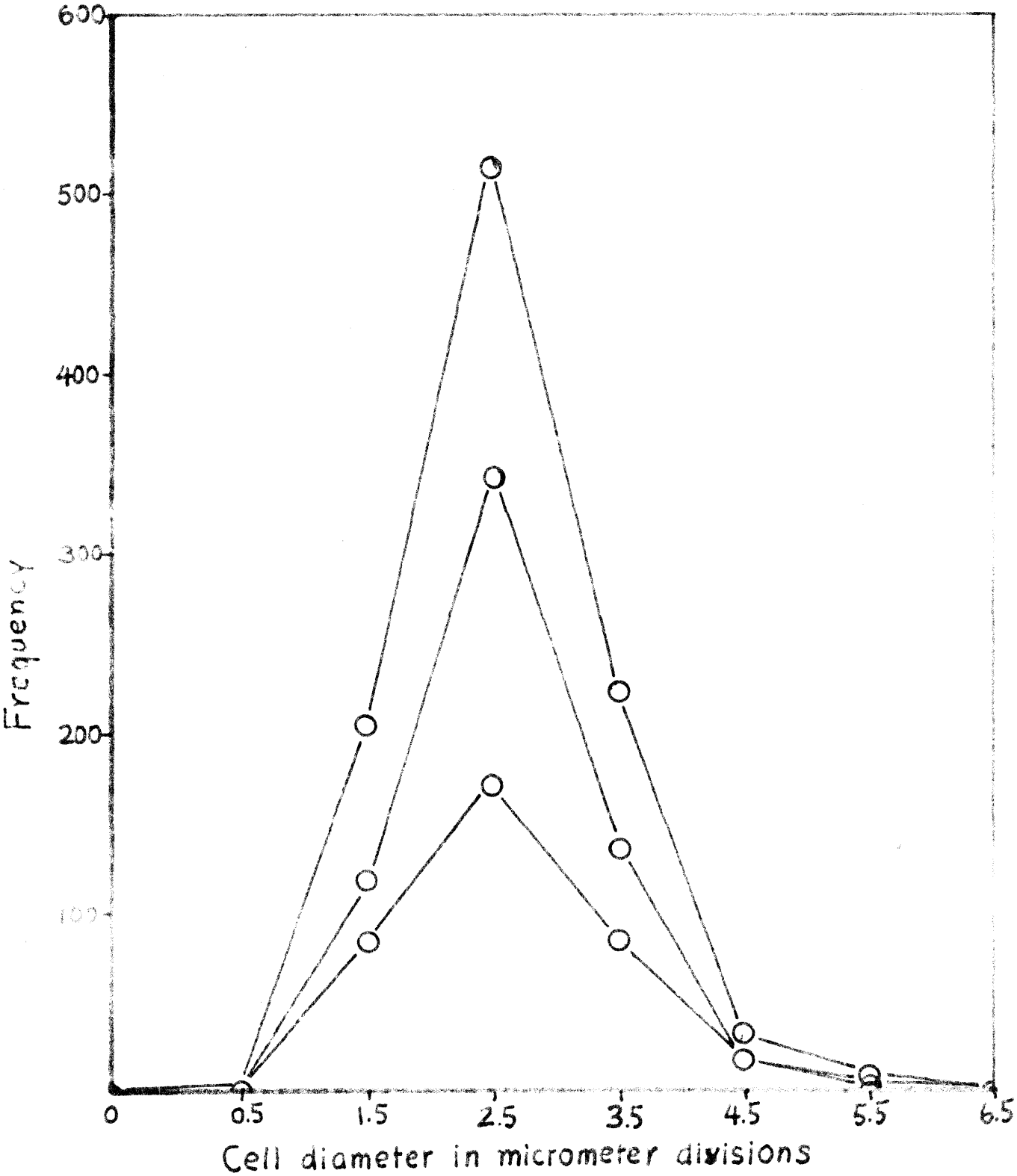


FIG 3 CELL SIZE OF DIFFERENT CULTURES

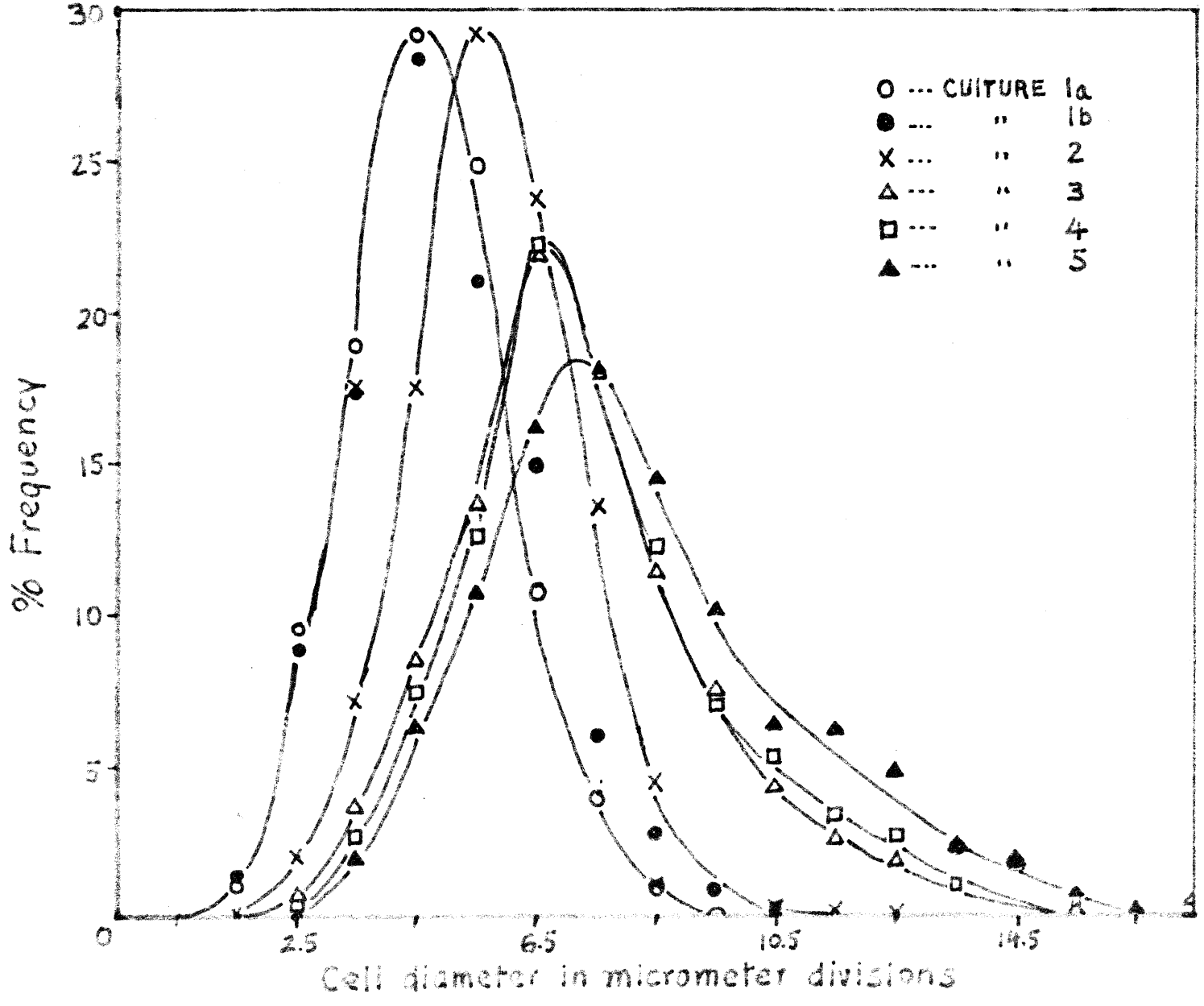


FIG. 4 AUXIN & CELL VOLUME.

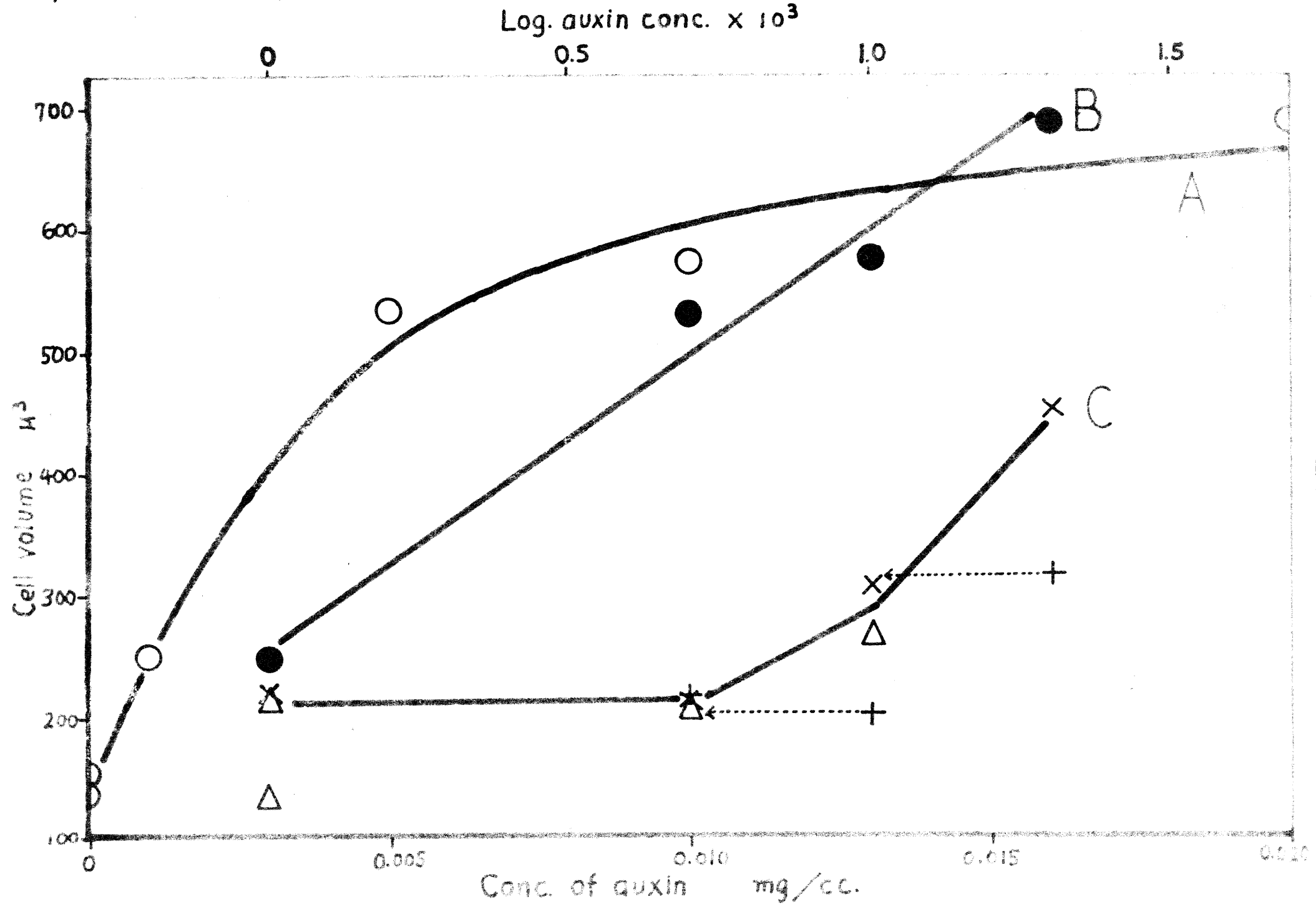


FIG. 5 PEA TEST

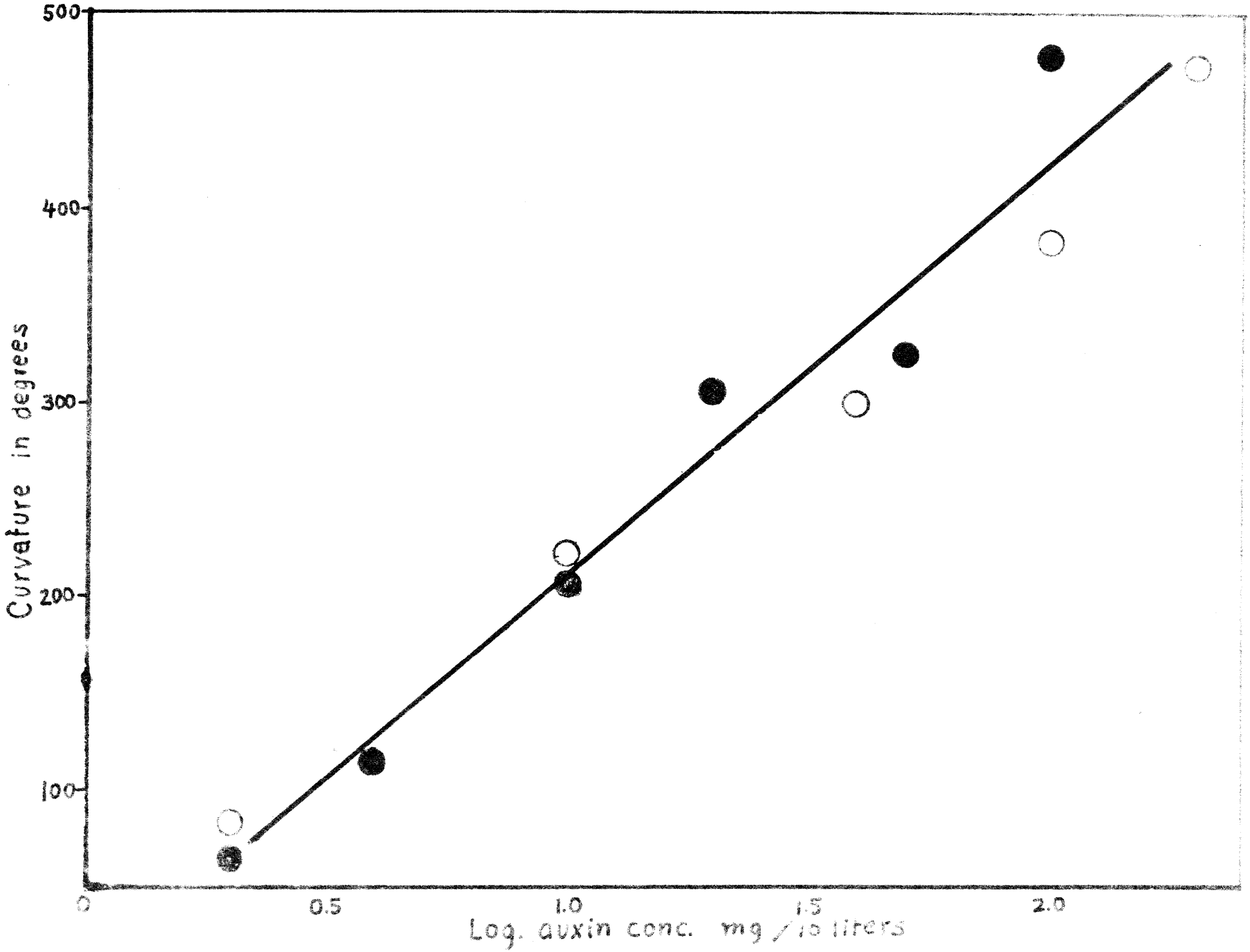


FIG. 6 CELL SURFACE, CHLOROPHYLL, & PHOTOSYNTHESIS.

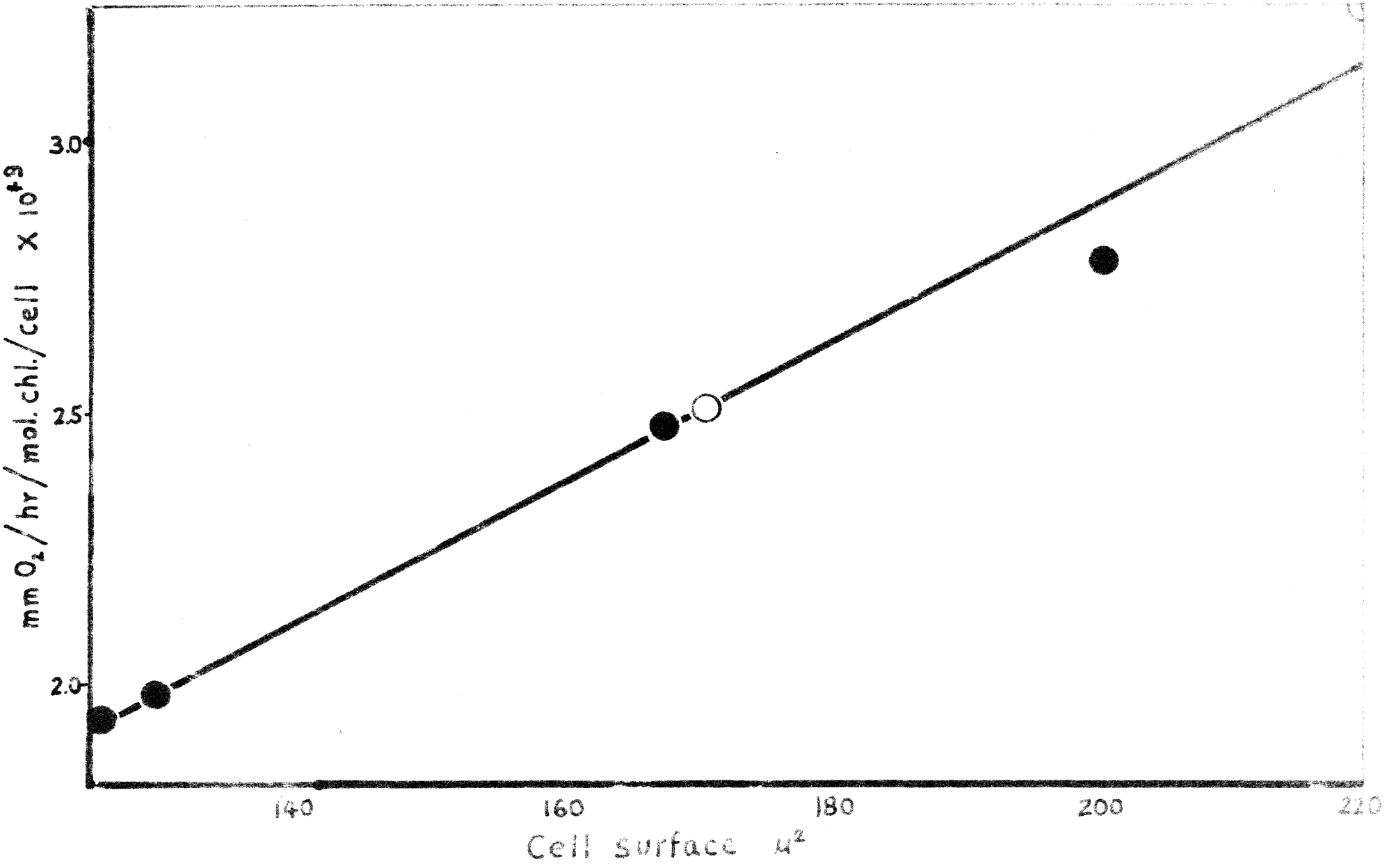


FIG. 7 NORMAL COURSE OF LEAF MOVEMENT.

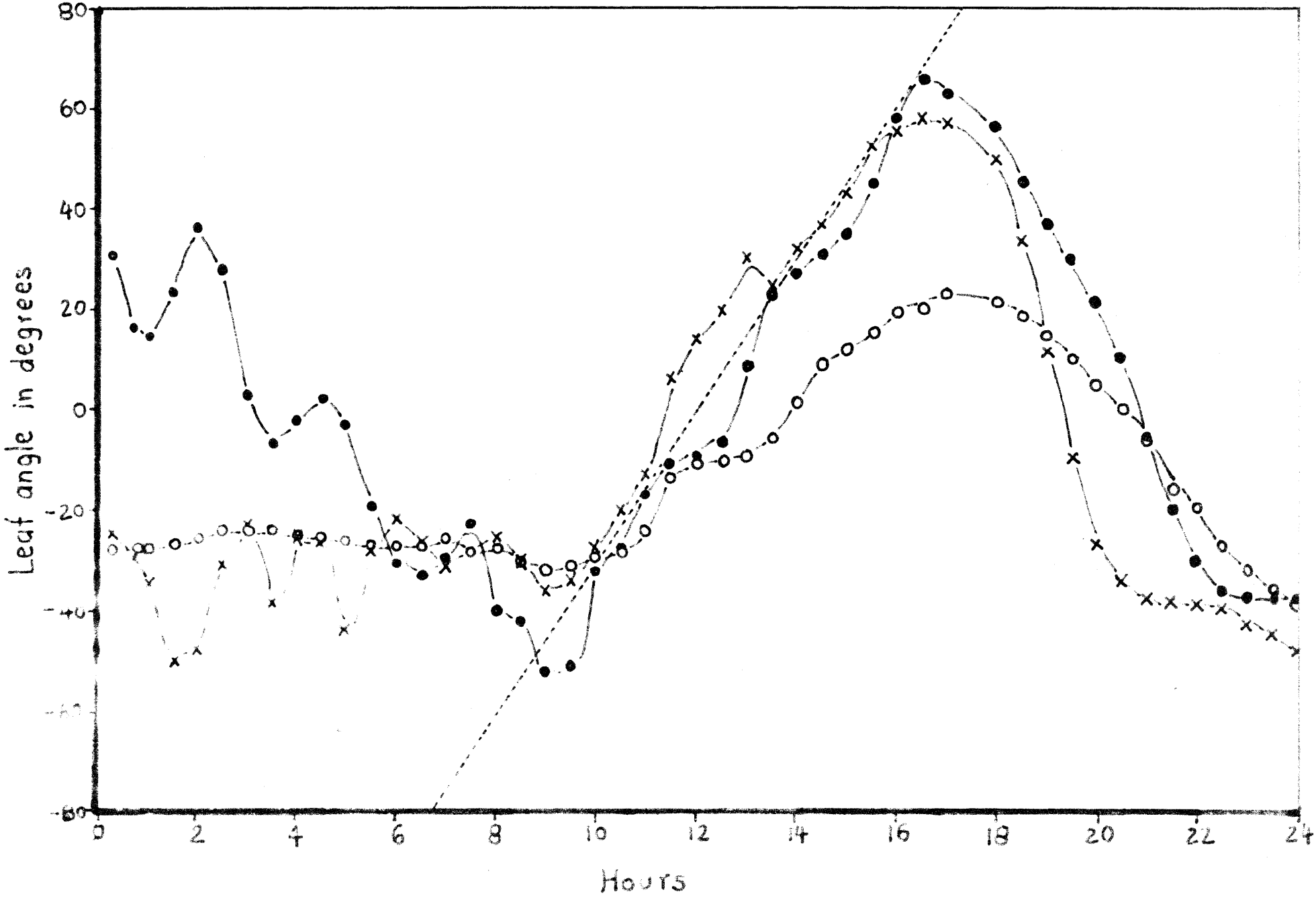


FIG. 8 LEAF MOVEMENT IN DIFFUSED LIGHT.

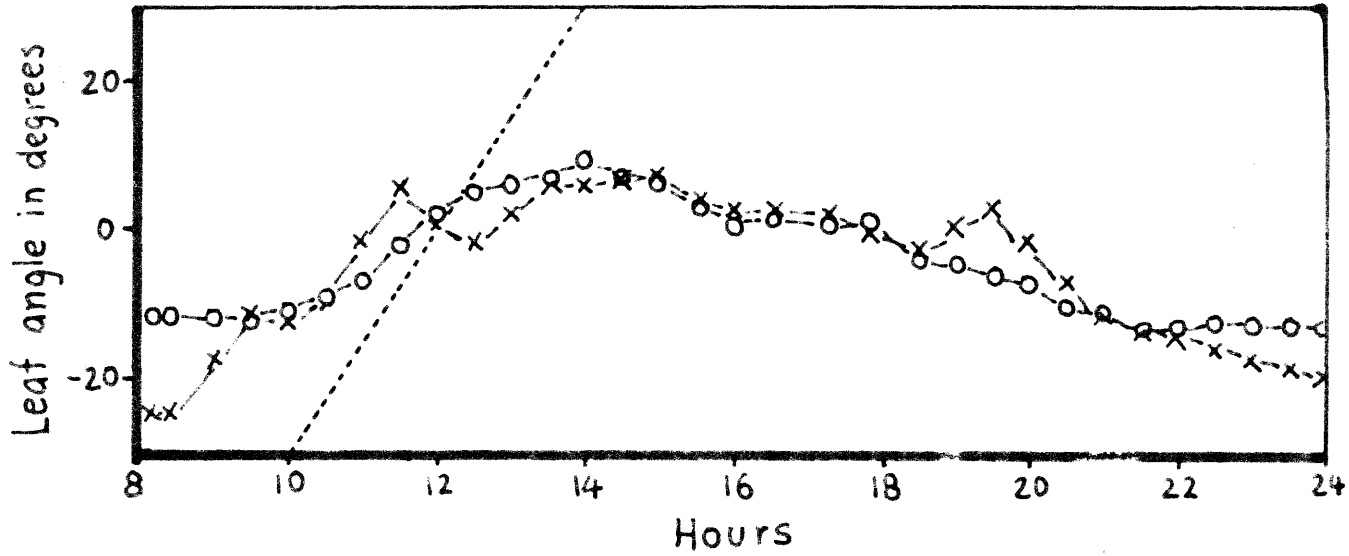


FIG. 9 MOVEMENT OF PETIOLE.

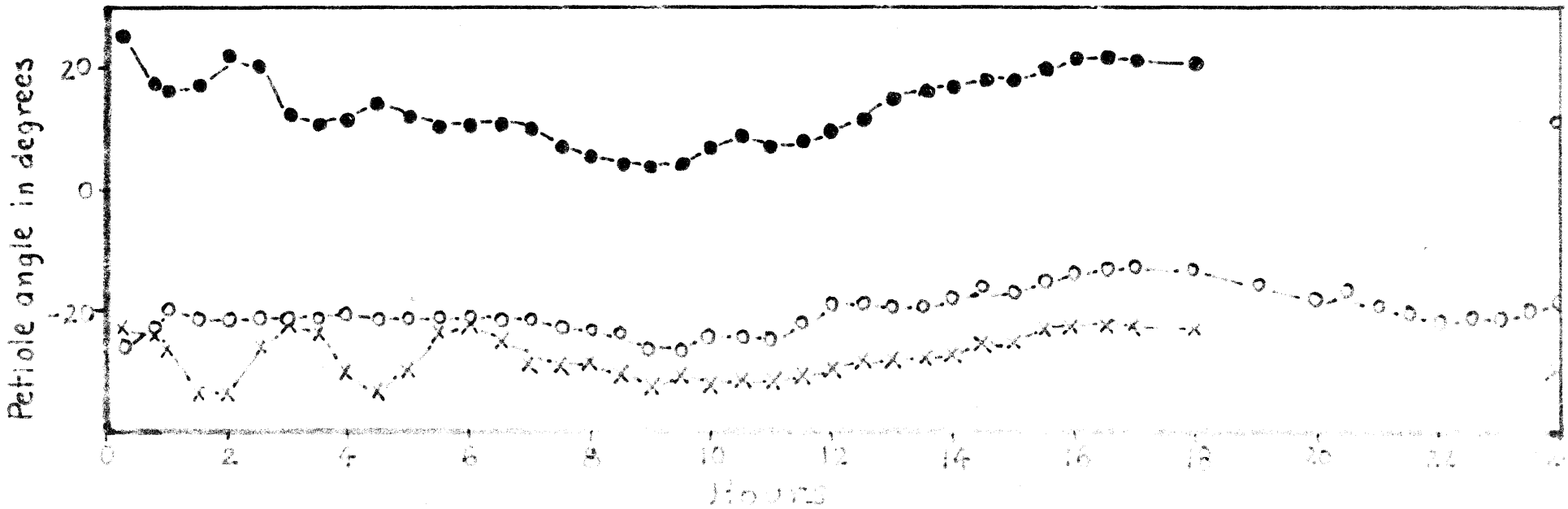
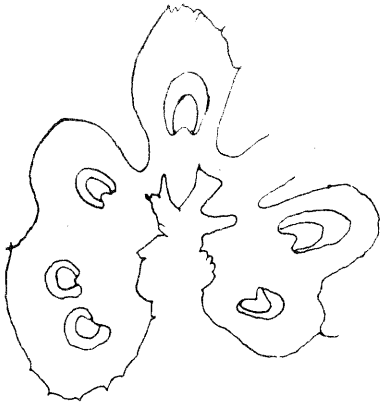


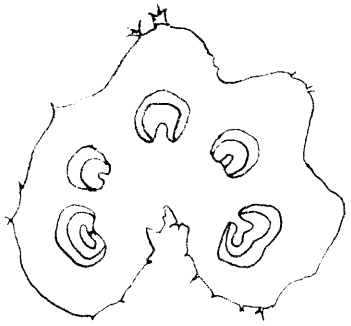
FIG. 12 STRUCTURE OF LAMINAR JOINT.

c.s. at different distances from tip (app.)

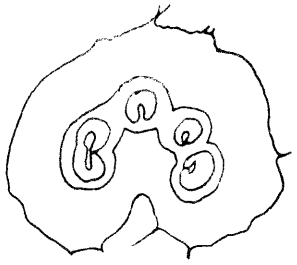
Leaf insertion



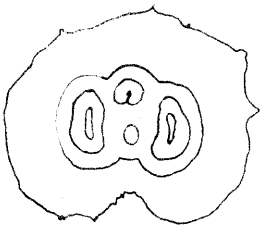
1 mm.



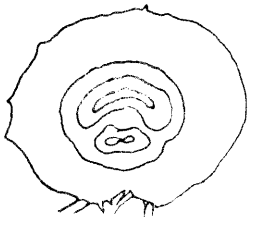
2 mm.



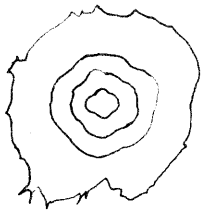
2.5 mm.



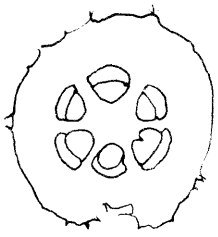
3 mm.



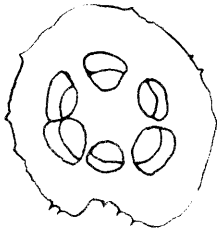
3.5 mm.



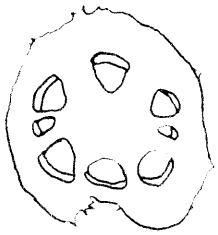
4 mm.



6 mm.



9 mm.



Petiole.

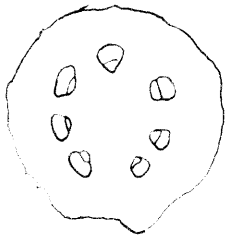


FIG. 13 CHANGES IN LENGTH OF 2 SIDES OF LAMINAR JOINT.

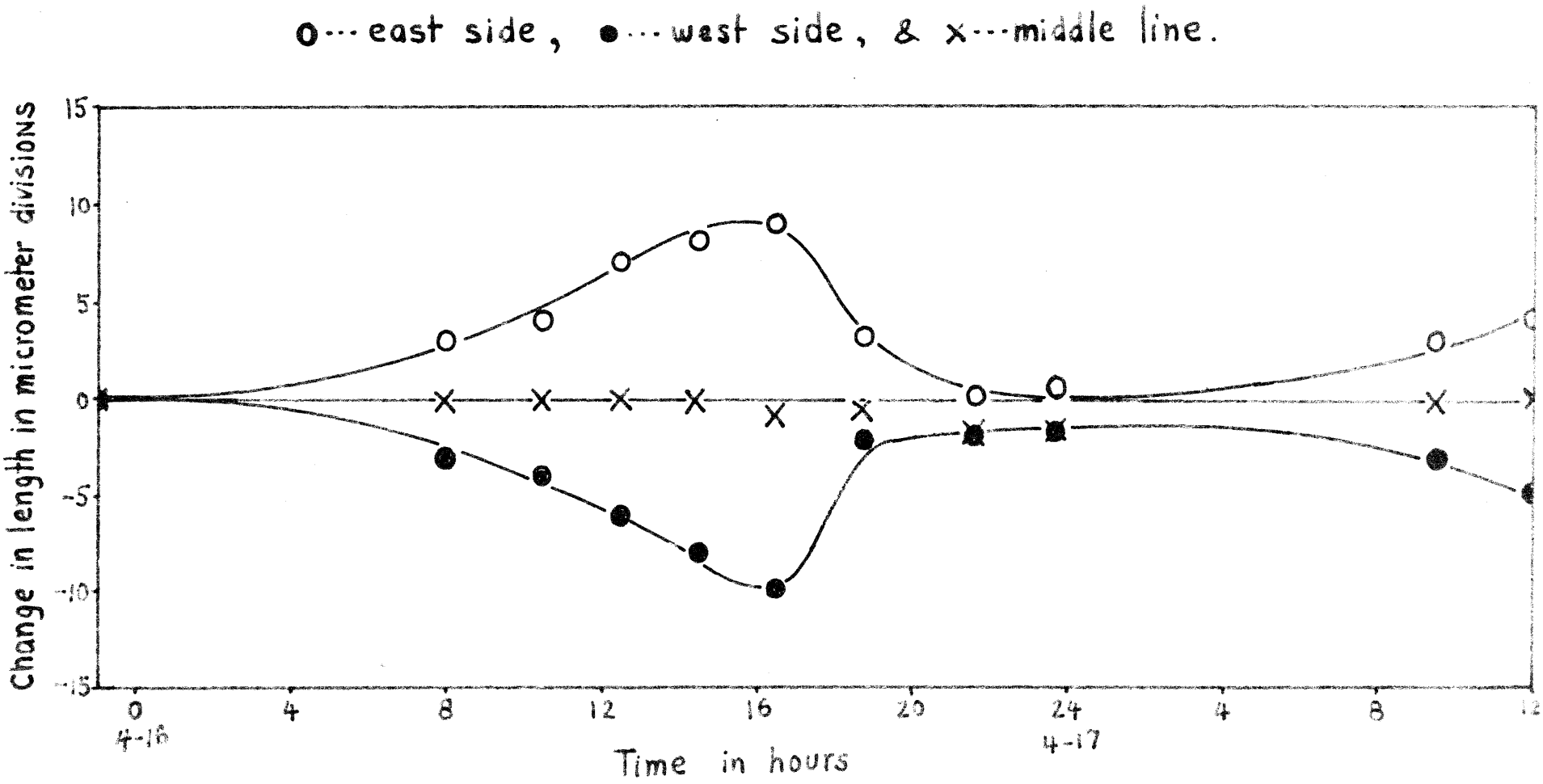
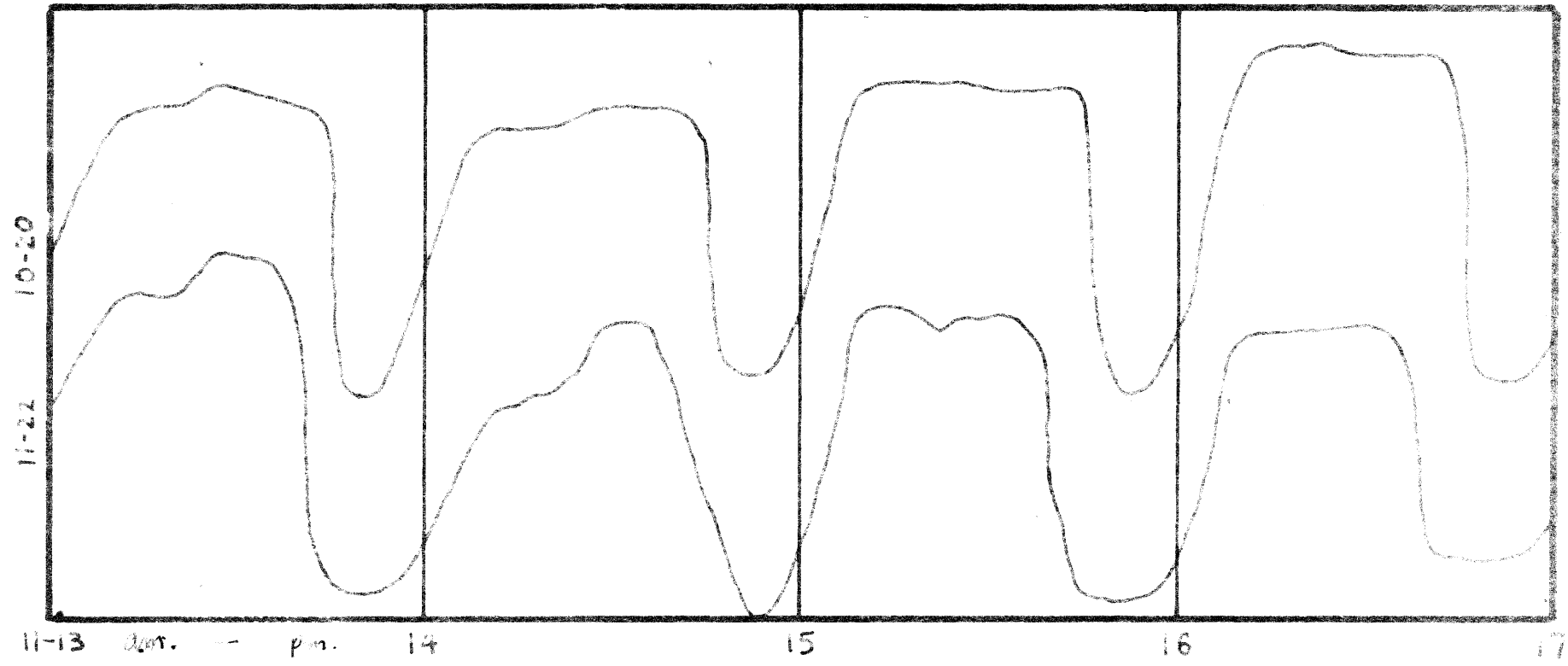


FIG. 14 LEAF MOVEMENT UNDER NORMAL CONDITIONS (Expt. 7)



(Expt. 1)

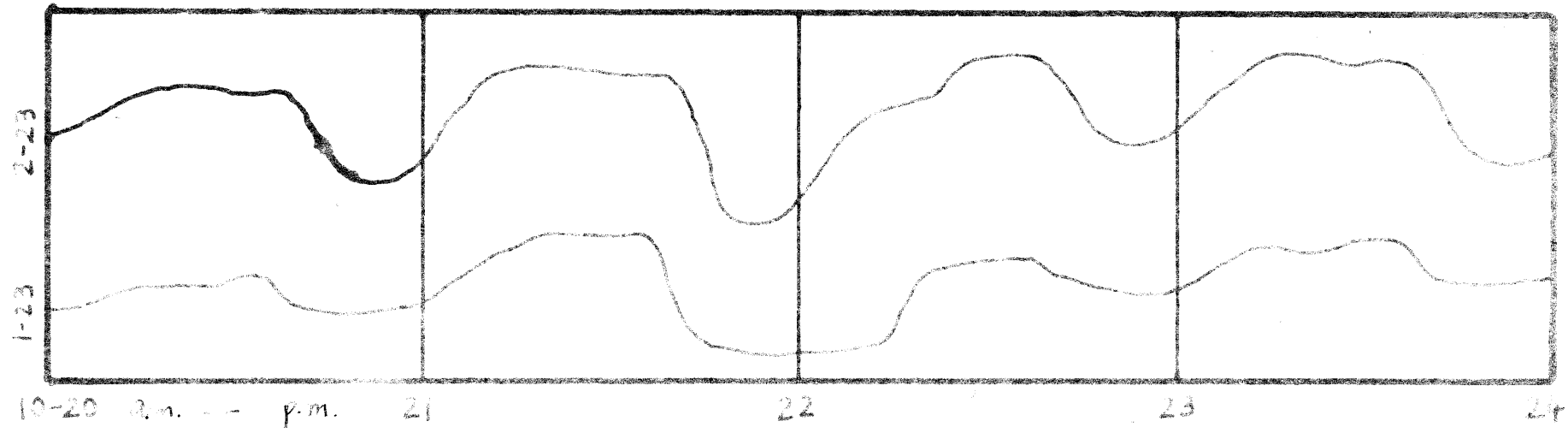


FIG 15 LEAF MOVEMENT UNDER NORMAL CONDITIONS

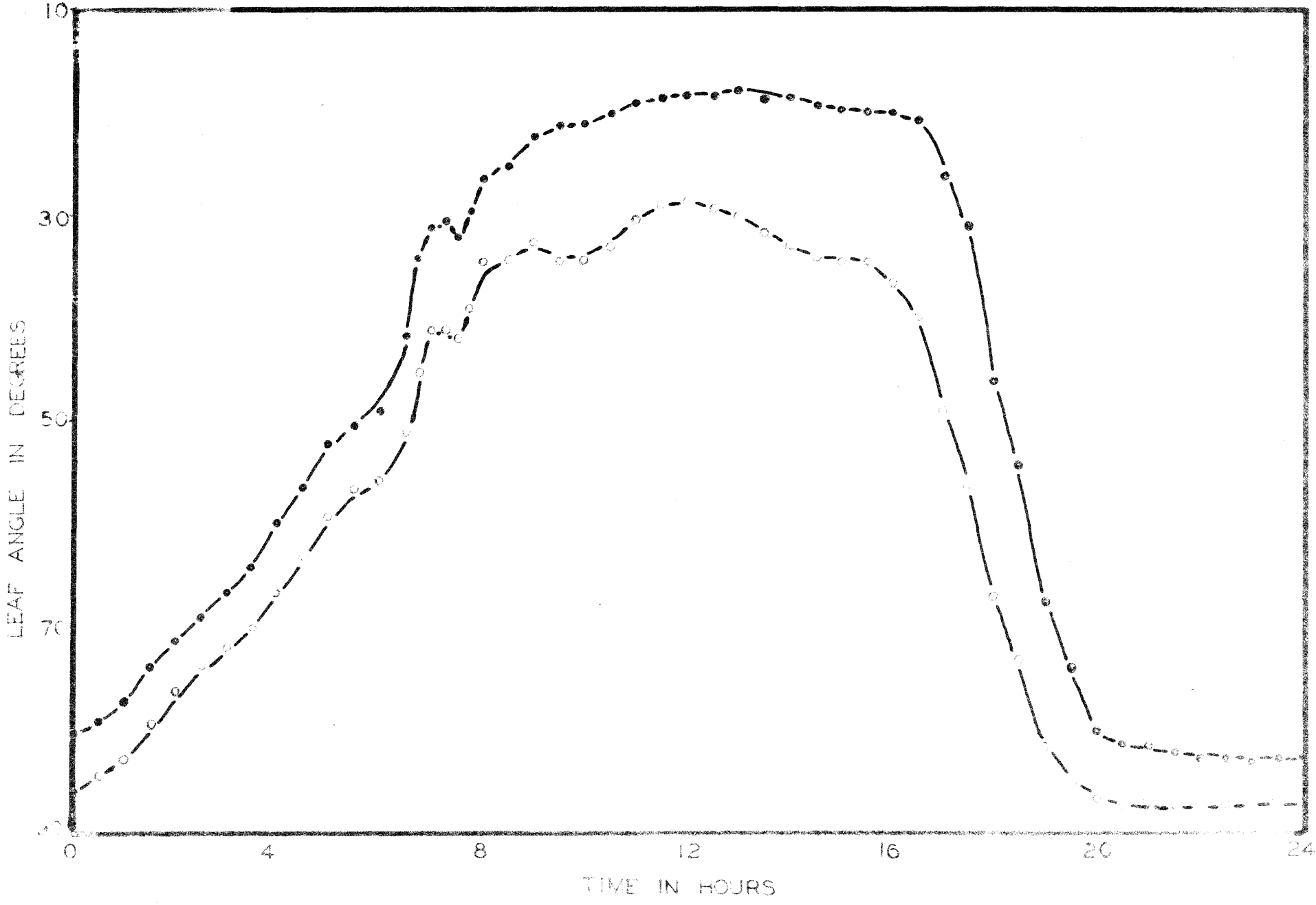


FIG 16 LEAF MOVEMENT IN CONSTANT LIGHT

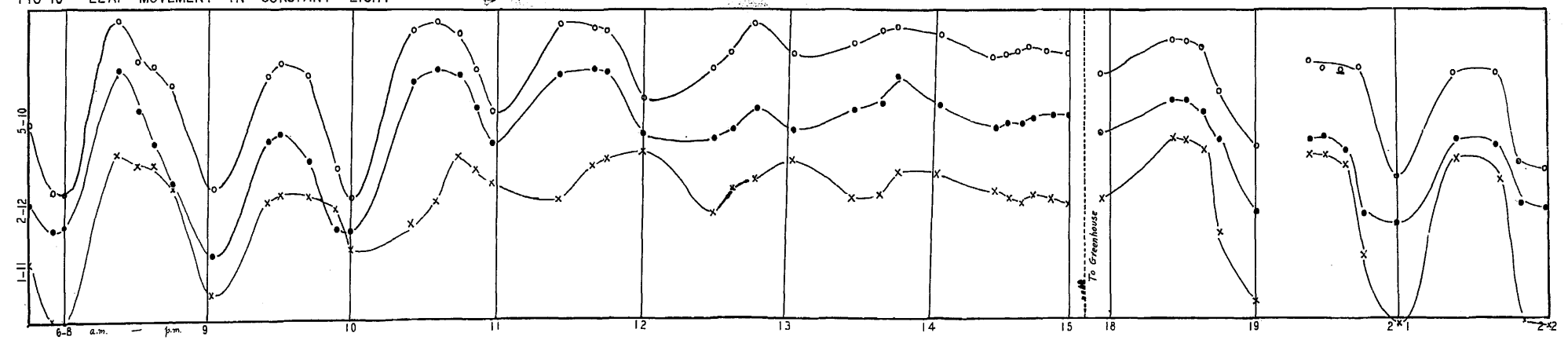


FIG. 17 LEAF MOVEMENT IN CONSTANT LIGHT (Expt. 9)

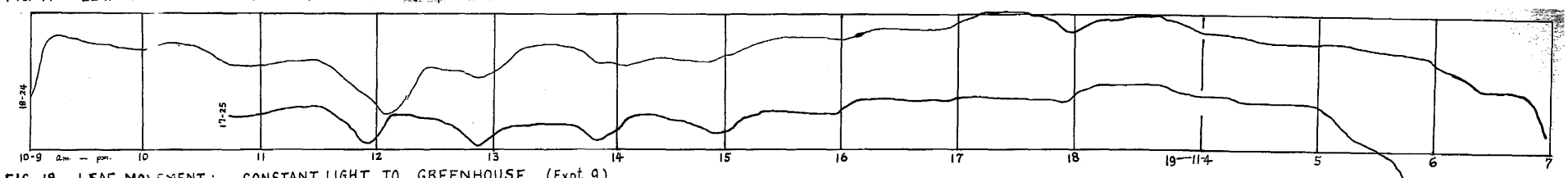


FIG. 18 LEAF MOVEMENT: CONSTANT LIGHT TO GREENHOUSE (Expt. 9)

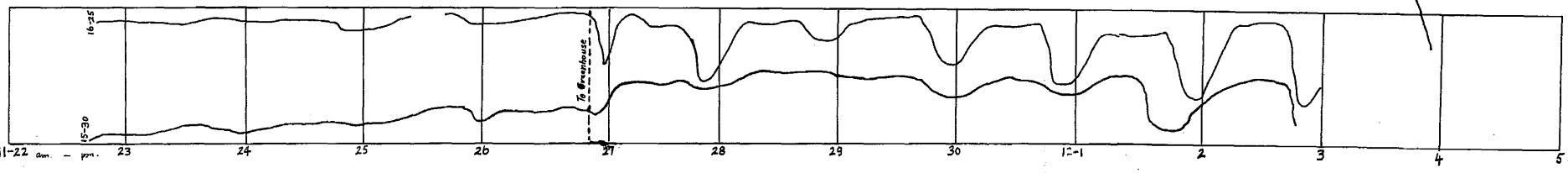


FIG. 19 LEAF MOVEMENT IN DARKNESS (Expt. 12 & 13)

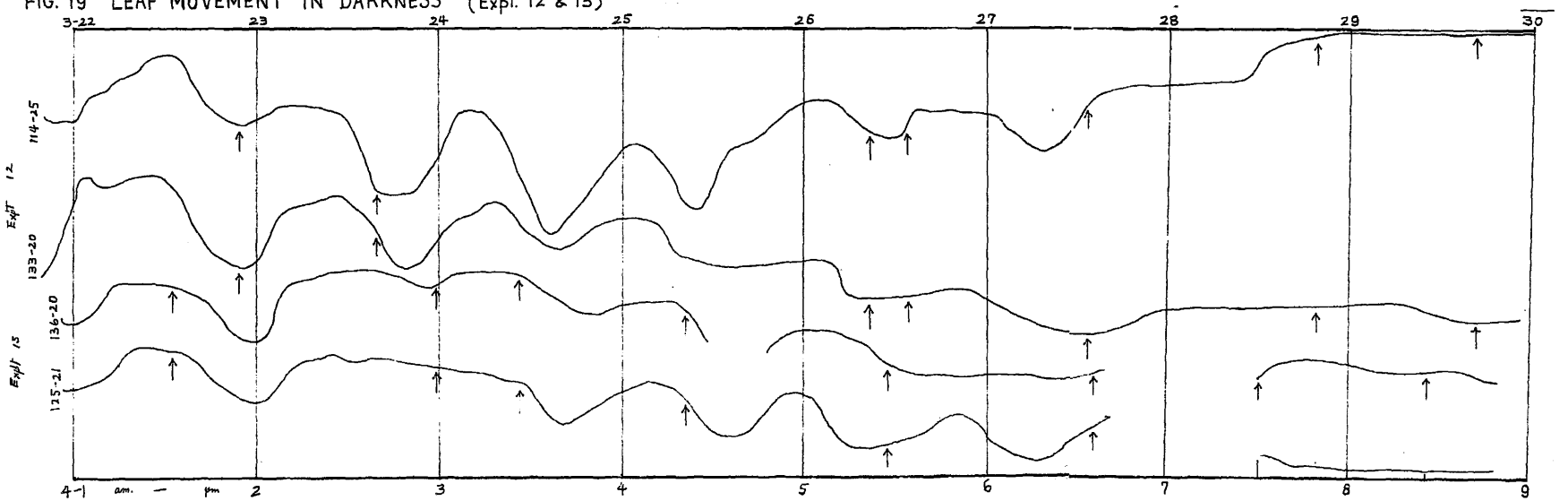


FIG. 20 MOVEMENT OF LEAVES OF DIFFERENT AGES

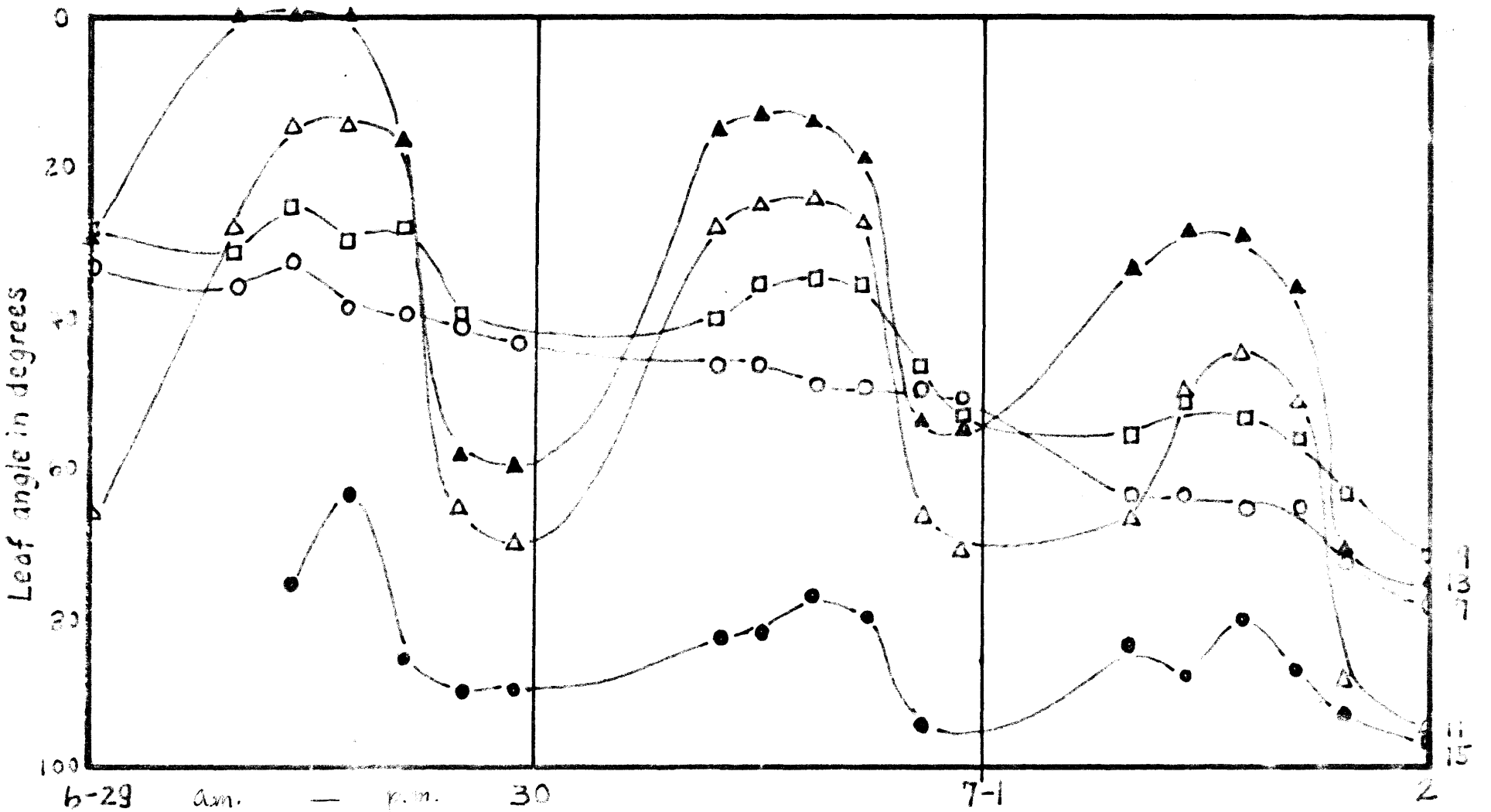


FIG. 22 DISTRIBUTION OF GROWTH.

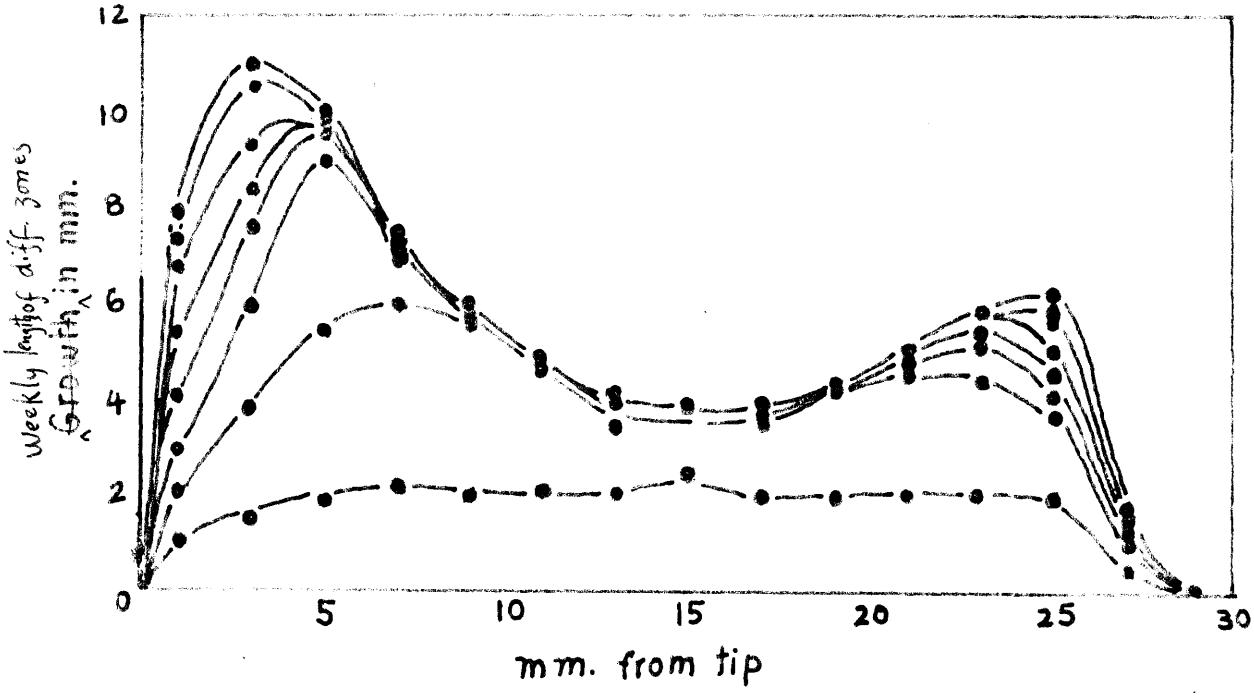


FIG 21 GROWTH OF PETIOLE.

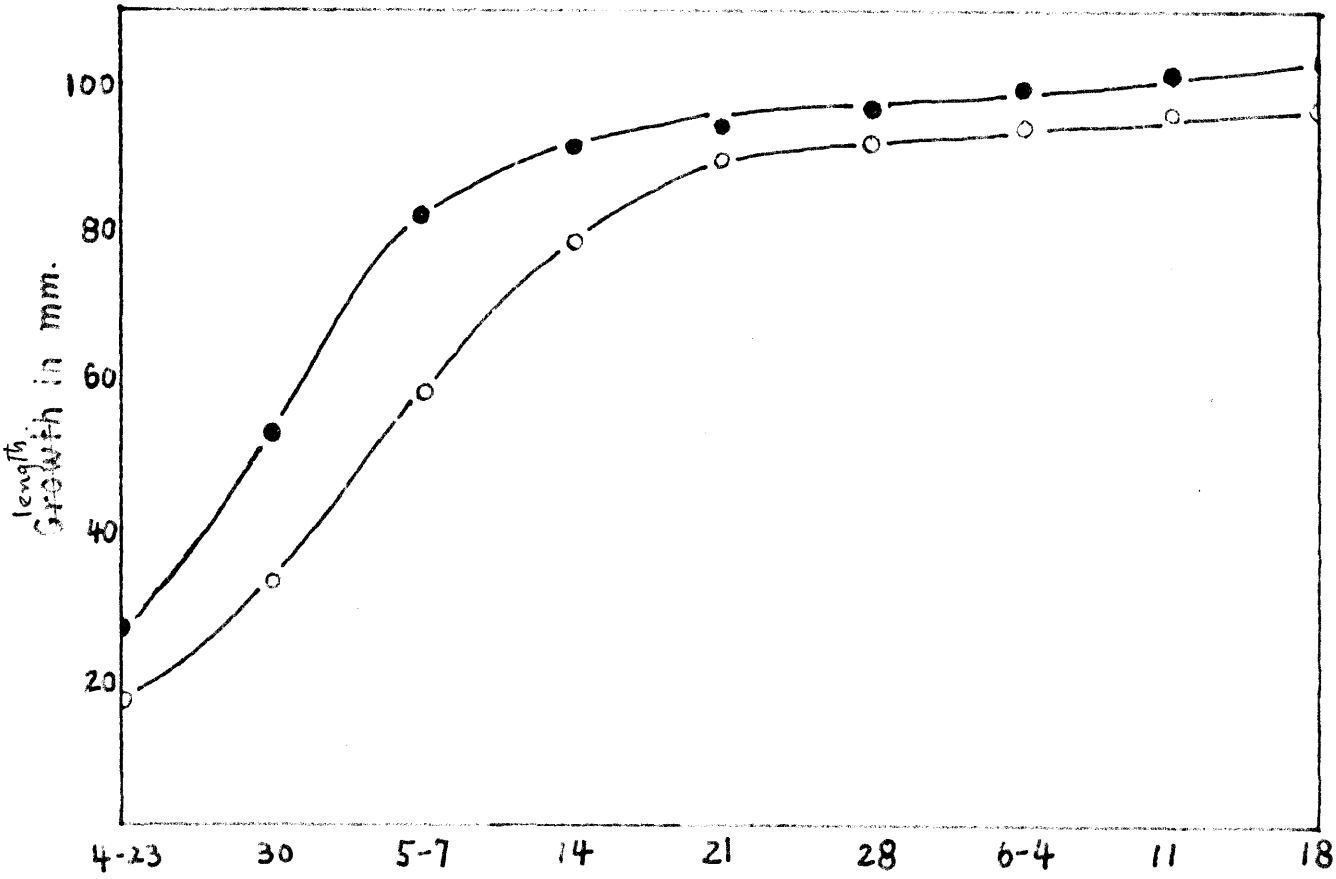


FIG. 23 AUXIN & GROWTH OF PETIOLE.

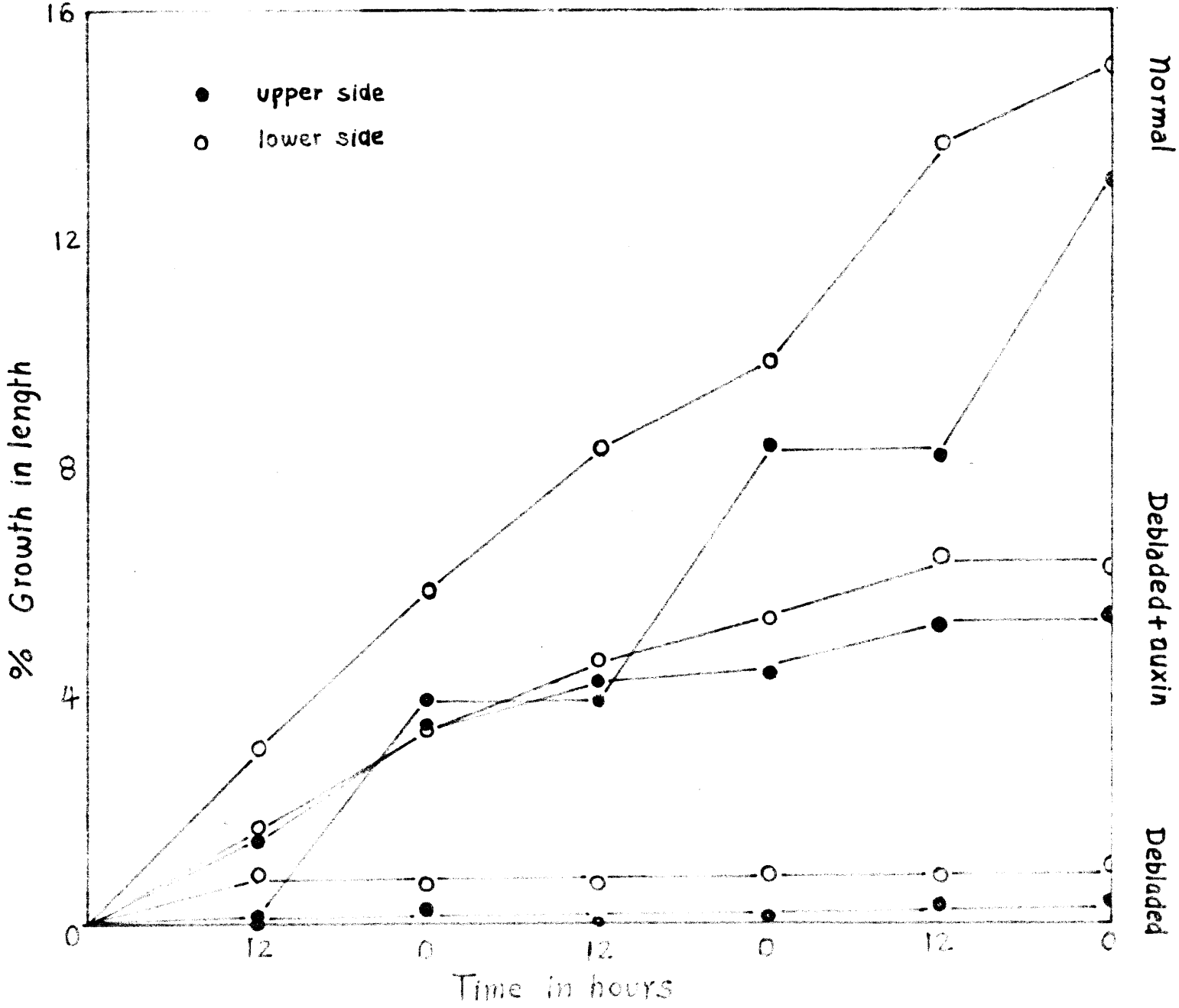


FIG 24 MOVEMENT OF DEBLADED LEAVES

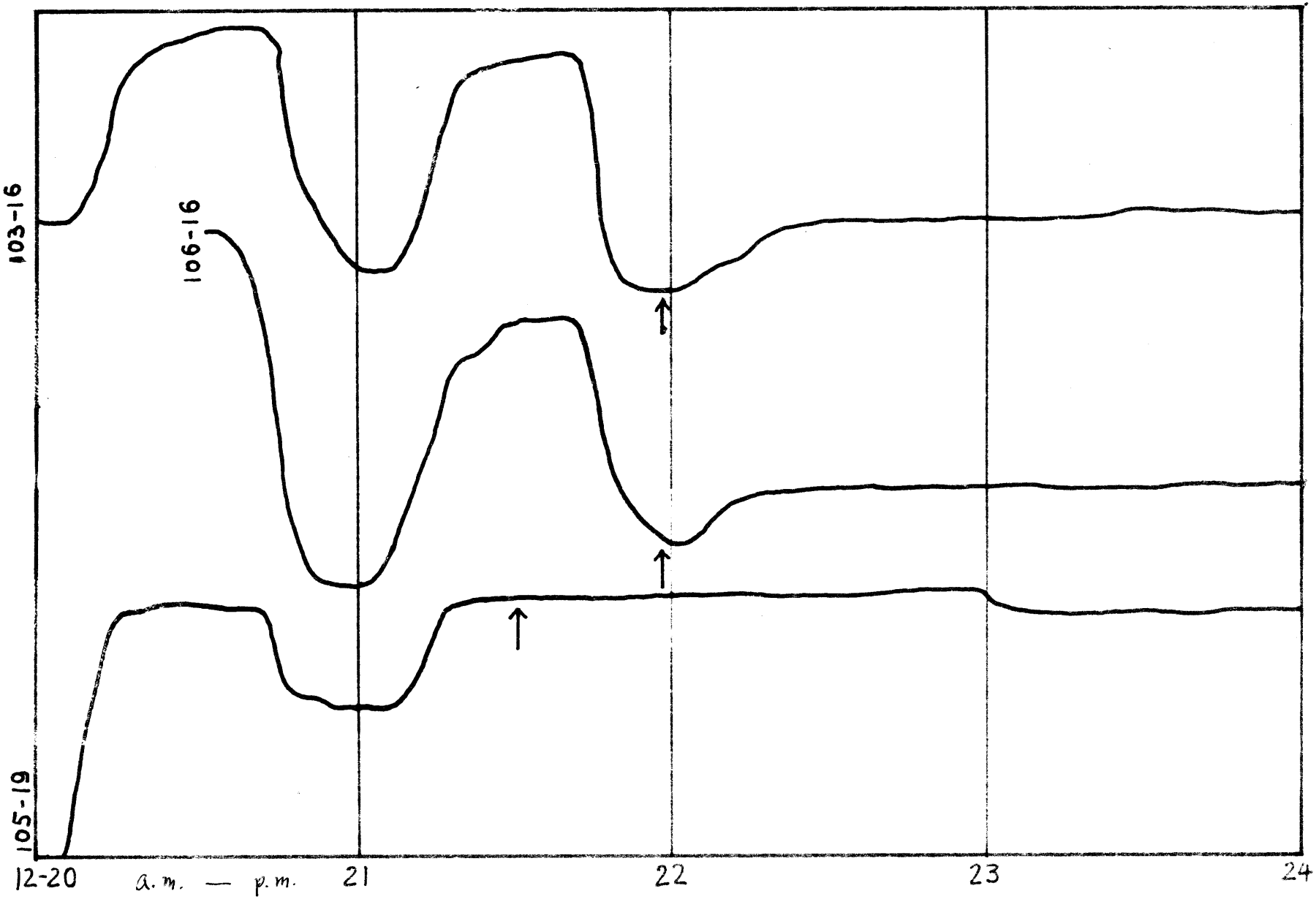


FIG 25 LEAF MOVEMENT, DEBLADE + AUXIN.

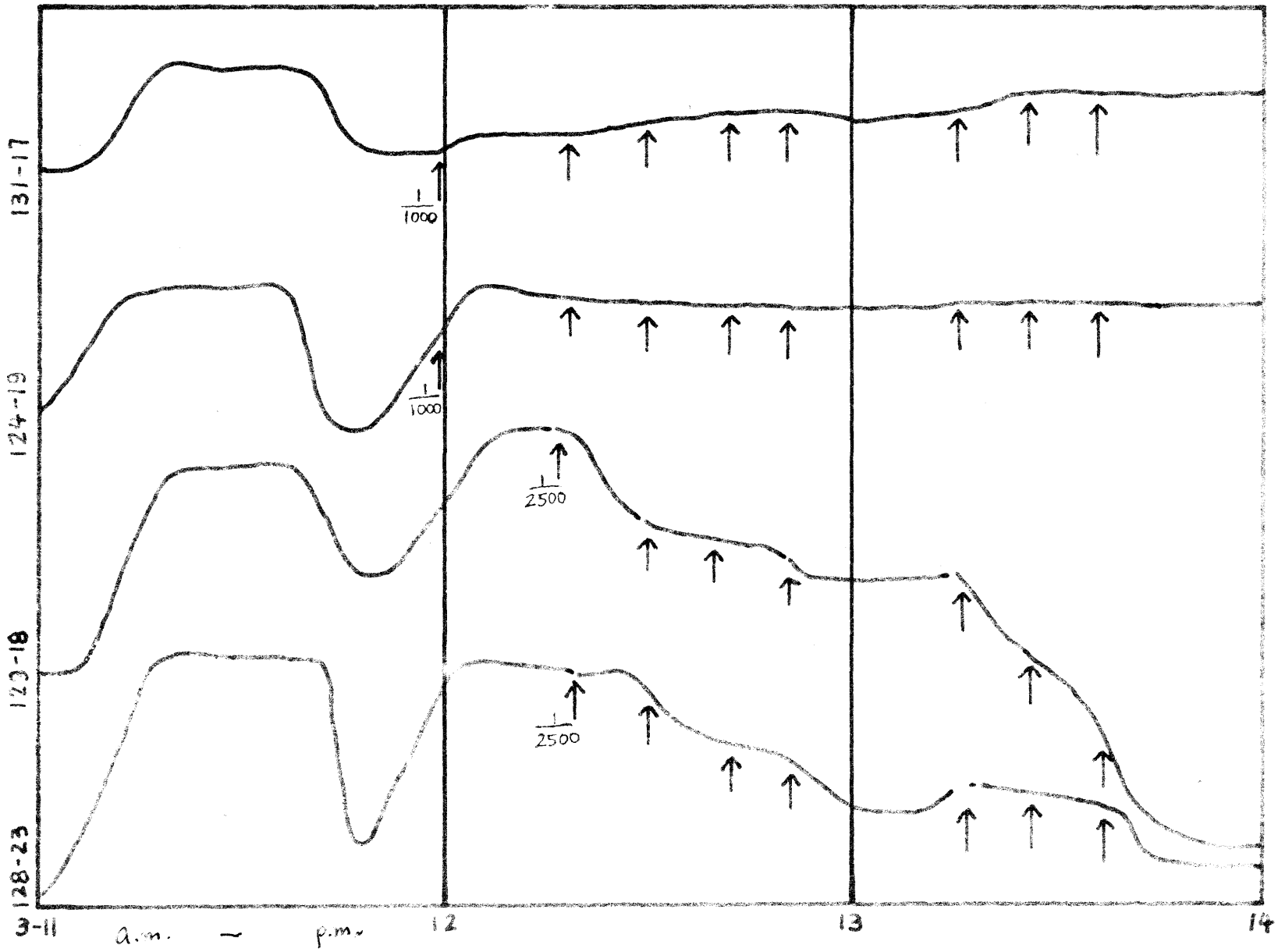


FIG 26 DIFFERENTIAL GROWTH & MOVEMENT.

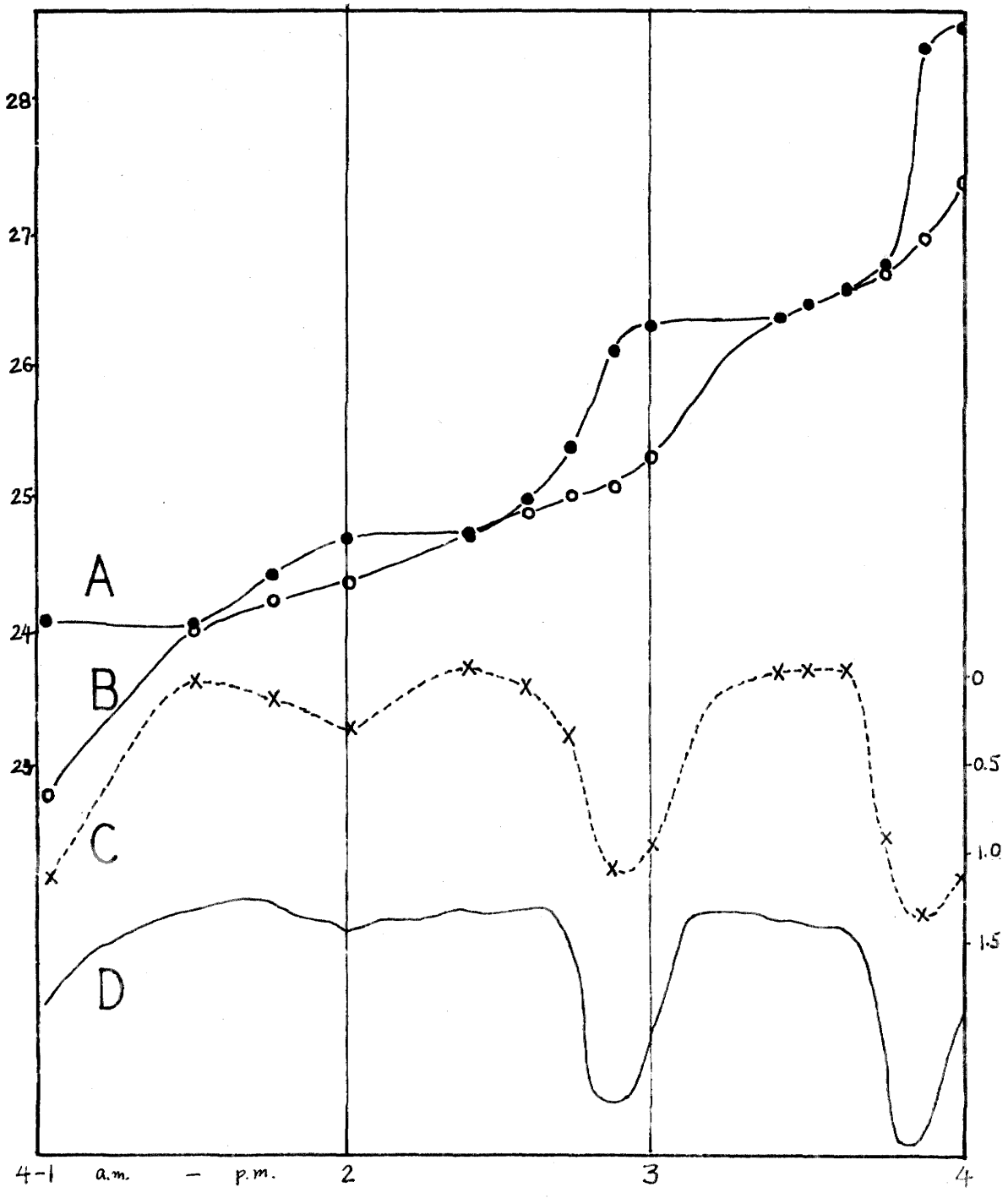


FIG. 27 FORM OF PAPAYA LEAF.

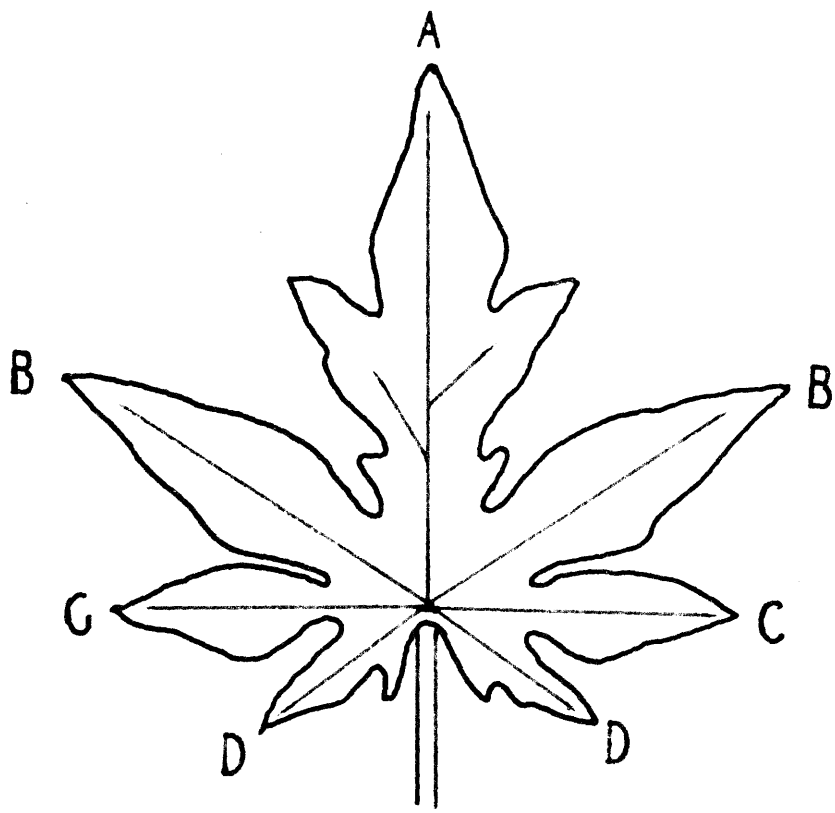
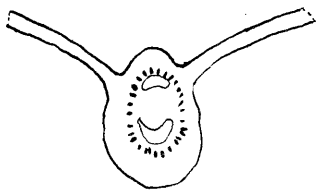
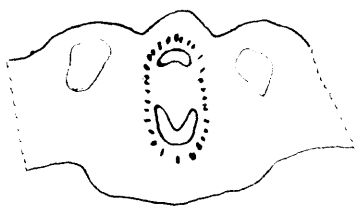


FIG. 28 STRUCTURE OF MIDRIB & PETIOLE

A

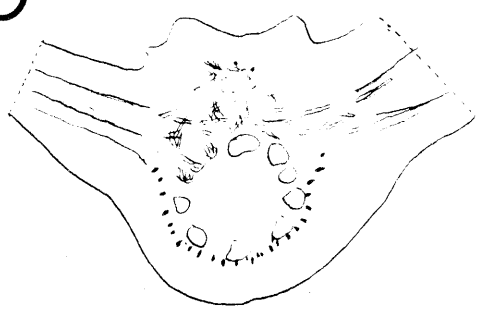


B

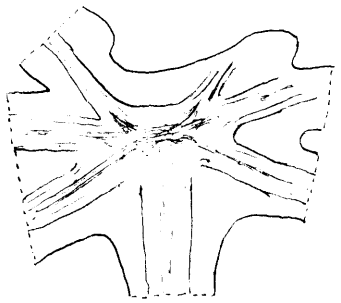


C

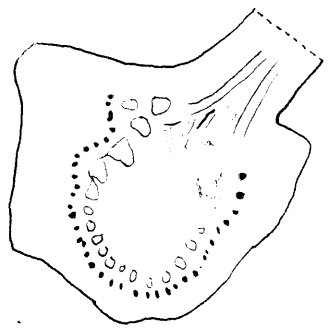
D



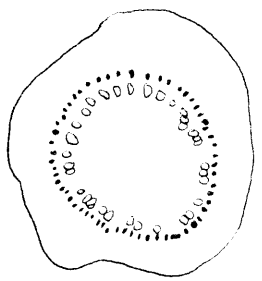
E



F



G



H

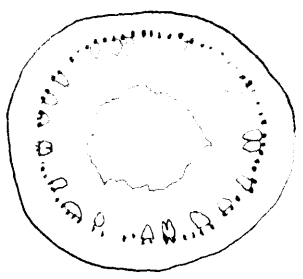
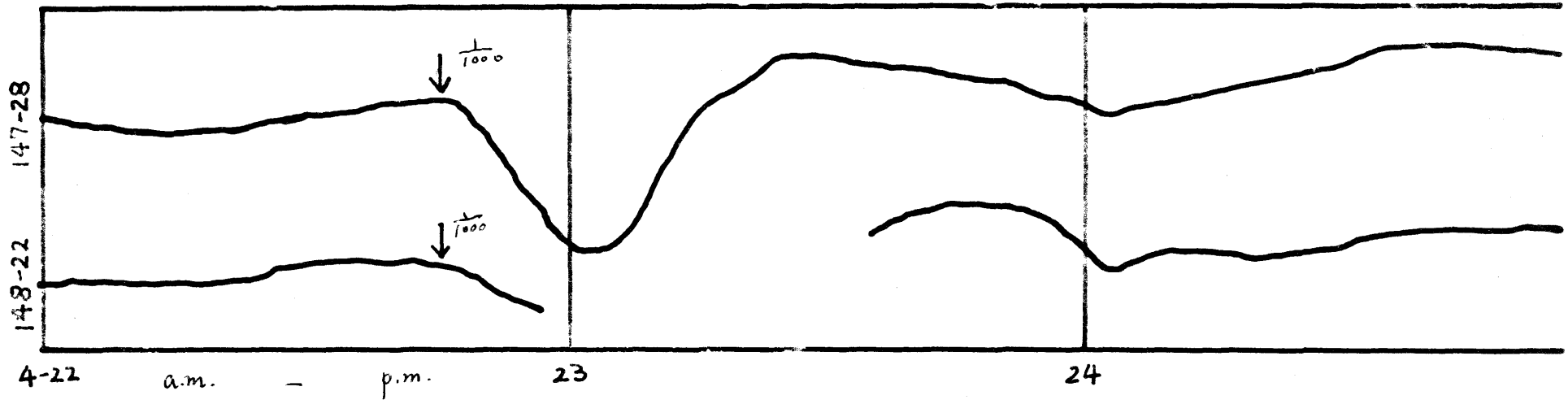


FIG 29 LEAF MOVEMENT INDUCED BY AUXIN IN CONSTANT LIGHT (Expt. 32)



(Expt. 36)

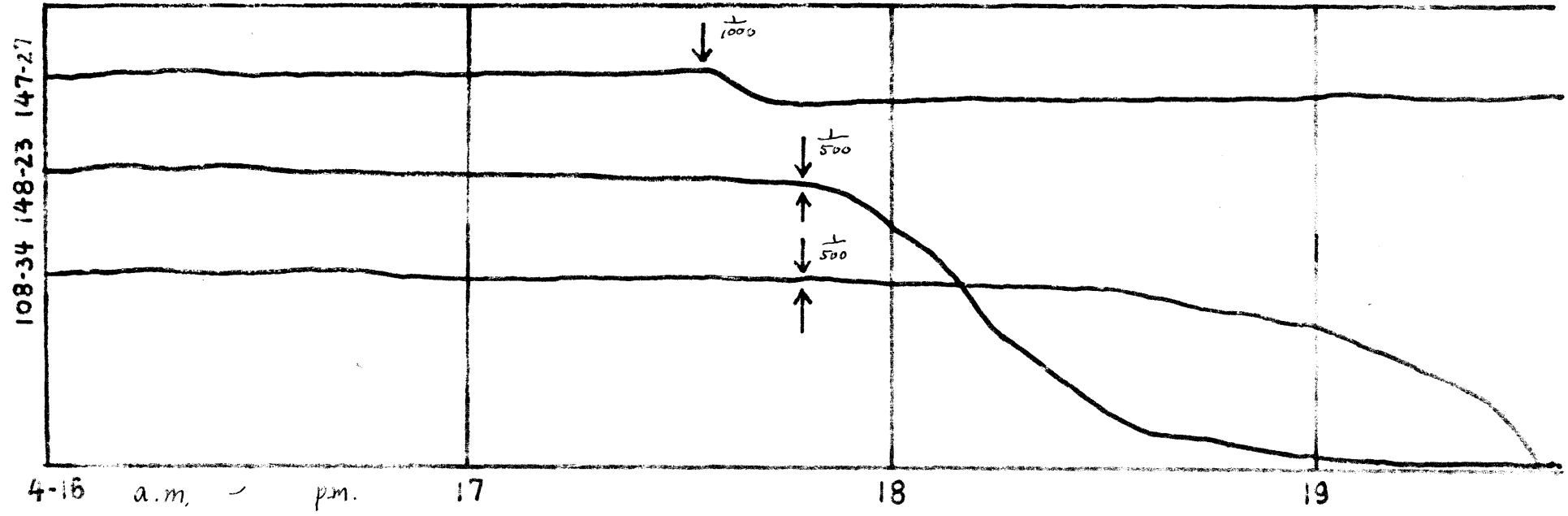


FIG 30 LEAF MOVEMENT INDUCED BY AUXIN IN GREENHOUSE

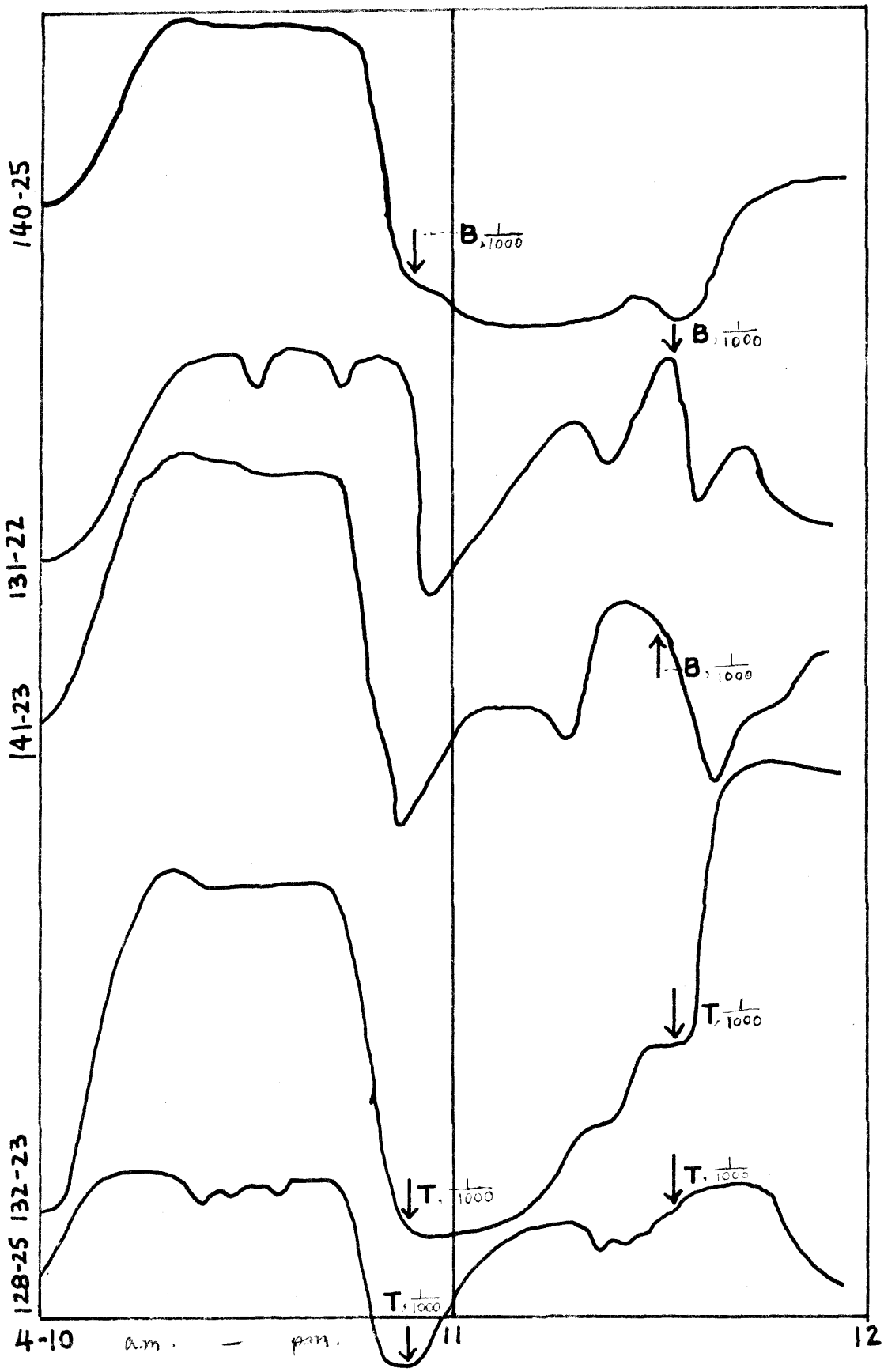


FIG 31 LEAF MOVEMENT INDUCED BY AUXIN IN GREENHOUSE

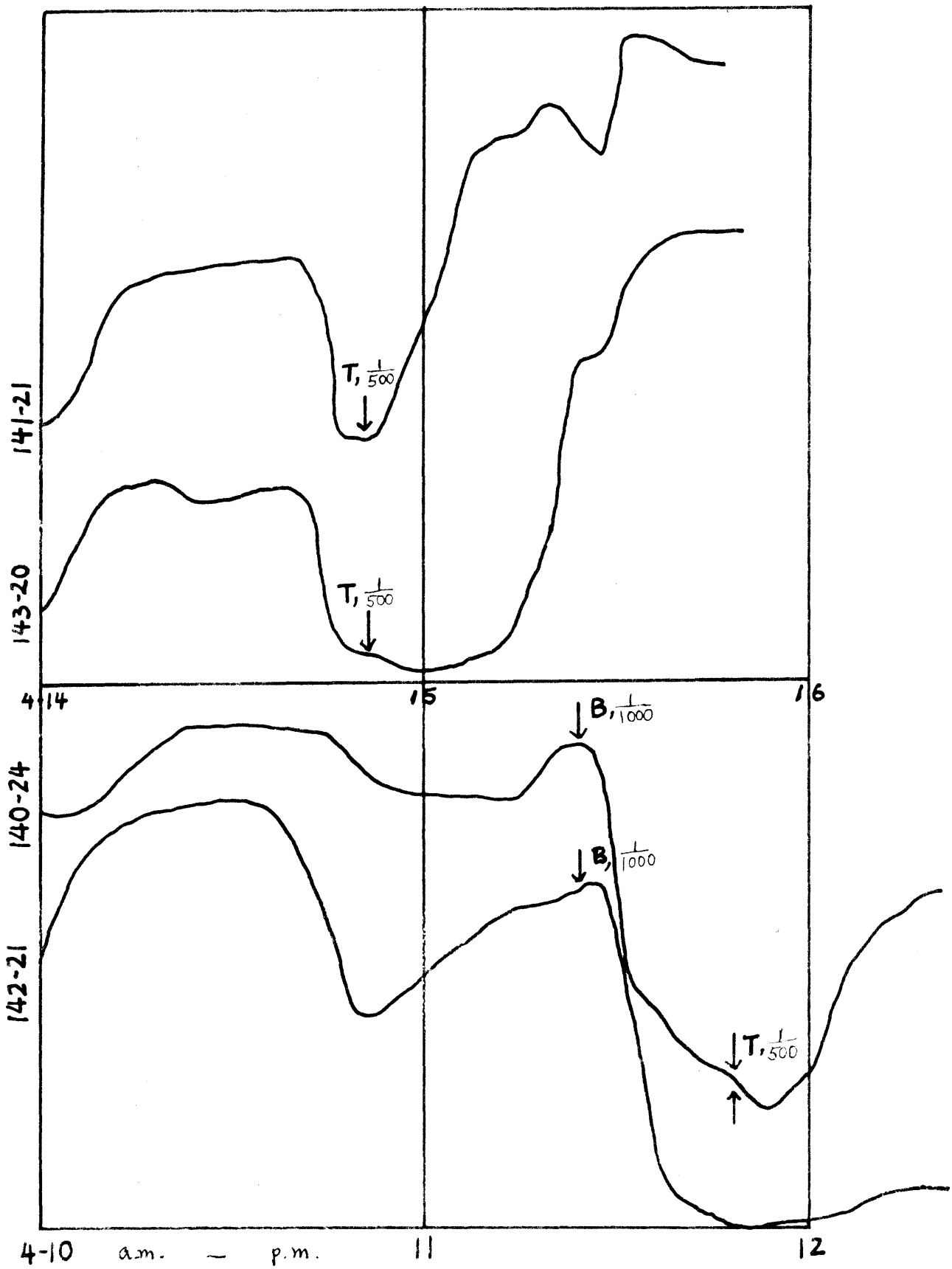
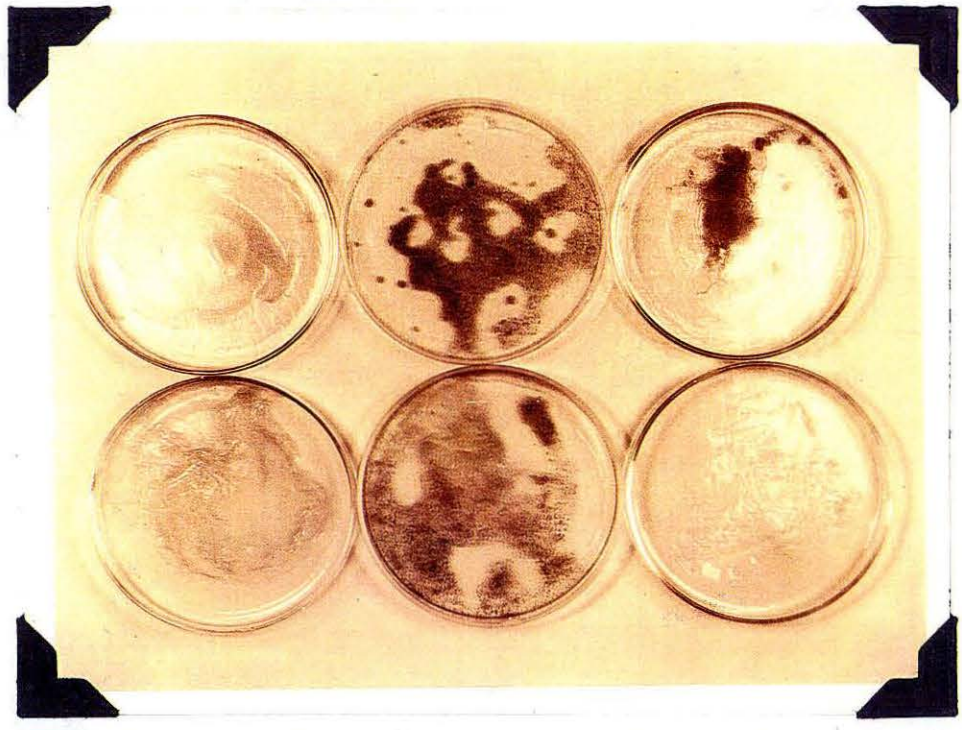


Plate I.

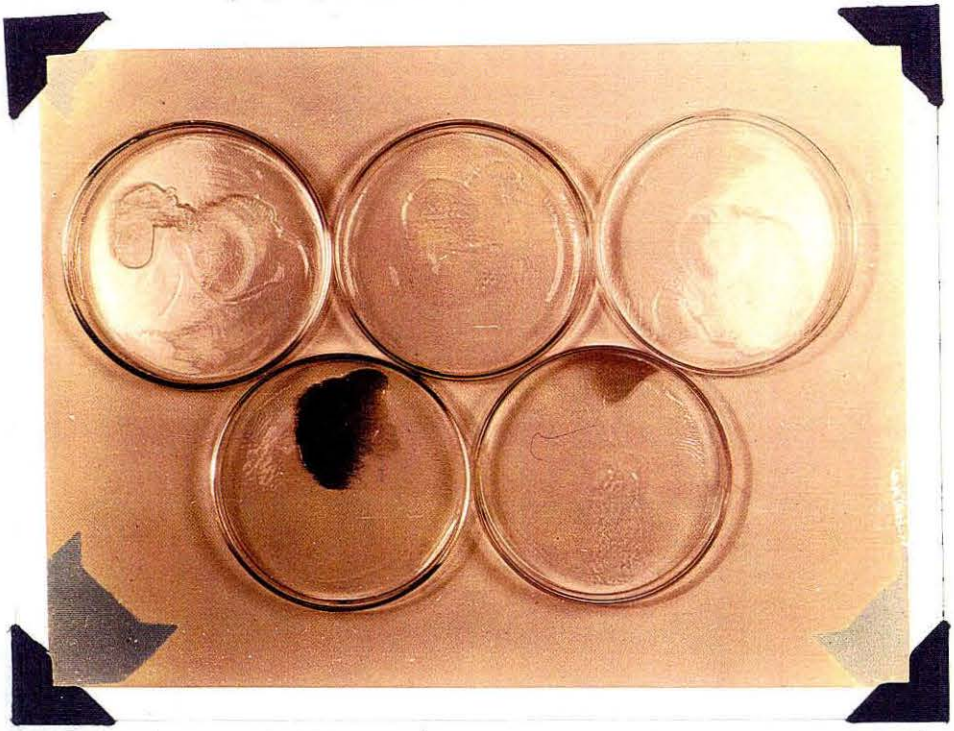
A. upper left ---------- control; lower left ---------- control.
upper middle - 0.0001 mg./c.c.; lower middle - 0.001 mg./c.c.
heteroauxin heteroauxin
upper right - 0.01 mg./c.c. ; lower right - 0.040 mg./c.c.
heteroauxin heteroauxin



B. upper left ---- 0.01 mg./c.c.; lower left ---- 0.005 mg. /c.c.
benzoic acid phenyl acetic acid.
upper middle -- 0.10 mg./c.c.; lower middle -- 0.010 mg./c.c.
benzoic acid phenyl acetic acid.
upper right --- 0.50 mg./c.c.; lower right --- 0.005 mg./c.c.
benzoic acid phenyl acetic acid.

Plate II.

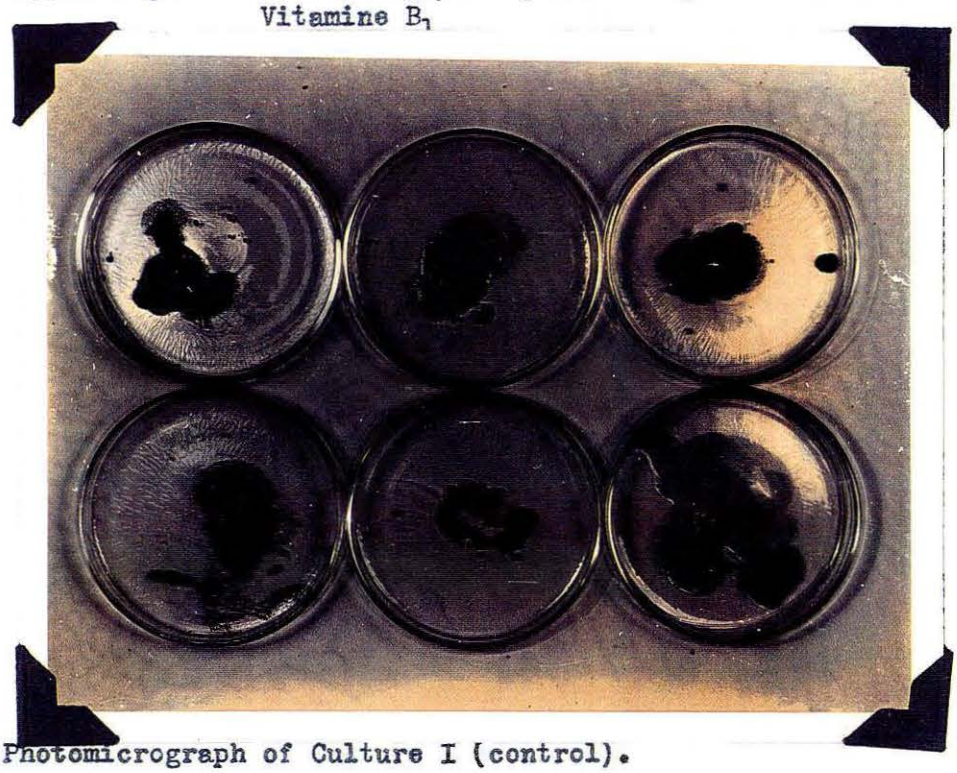
C. upper left ---- 0.05 mg./c.c.; lower left ---- 0.05 mg./c.c.
Ø-proprionic acid Ø-butyric acid.
upper middle -- 0.10 mg./c.c.; lower right --- 0.050 mg./c.c.
Ø-proprionic acid Ø-butyric acid.
upper right --- 0.50 mg./c.c.
Ø-proprionic acid.



D. upper left ---- 0.005 mg./c.c.; lower left ---- 0.05 mg./c.c.
cis-cinnamic acid trans-cinnamic acid.
upper middle -- 0.010 mg./c.c.; lower right --- 0.50 mg./c.c.
cis-cinnamic acid trans-cinnamic acid.
upper right --- 0.050 mg./c.c.;
cis-cinnamic acid.

Plate III.

E. upper left ---- 0.0001 mg./c.c.; lower left ---- 0.0001 mg./c.c. Vitamine B_1 Vitamine B_1.
upper middle -- 0.0005 mg./c.c.; lower middle -- 0.0005 mg./c.c. Vitamine B_1 Vitamine B_1.
upper right --- 0.0010 mg./c.c.; lower right --- control. Vitamine B_1



F. Photomicrograph of Culture I (control).

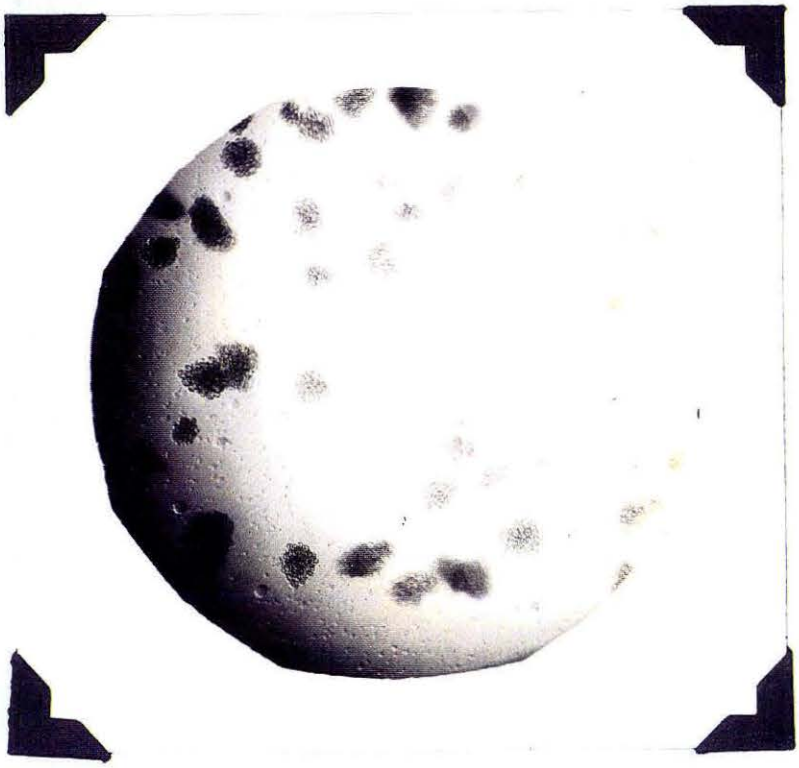
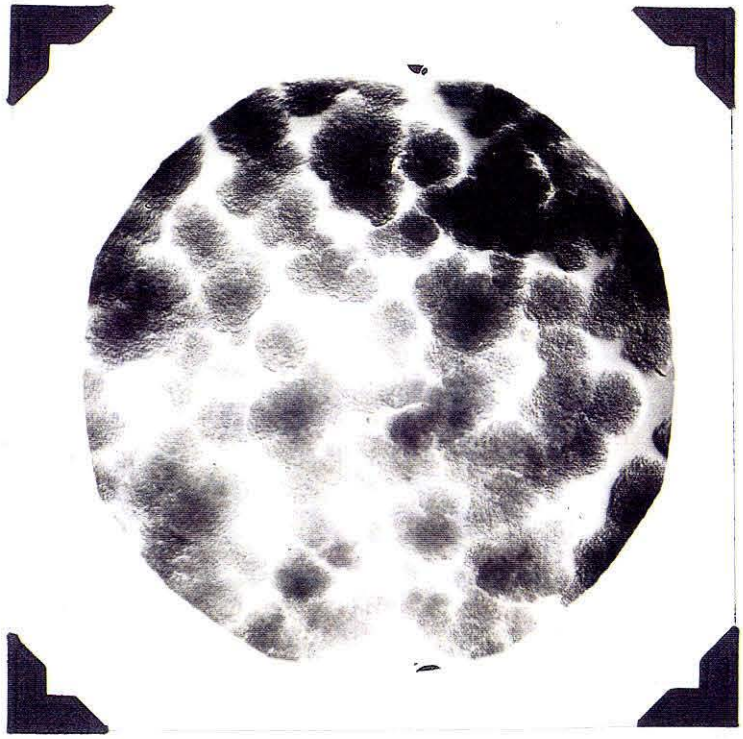


Plate IV.

G. Photomicrograph of Culture IIb (0.0001 mg./c.c. heteroauxin).



H. Photomicrograph of Culture IXa (0.0001 mg. /c.c. Vitamine B_1).

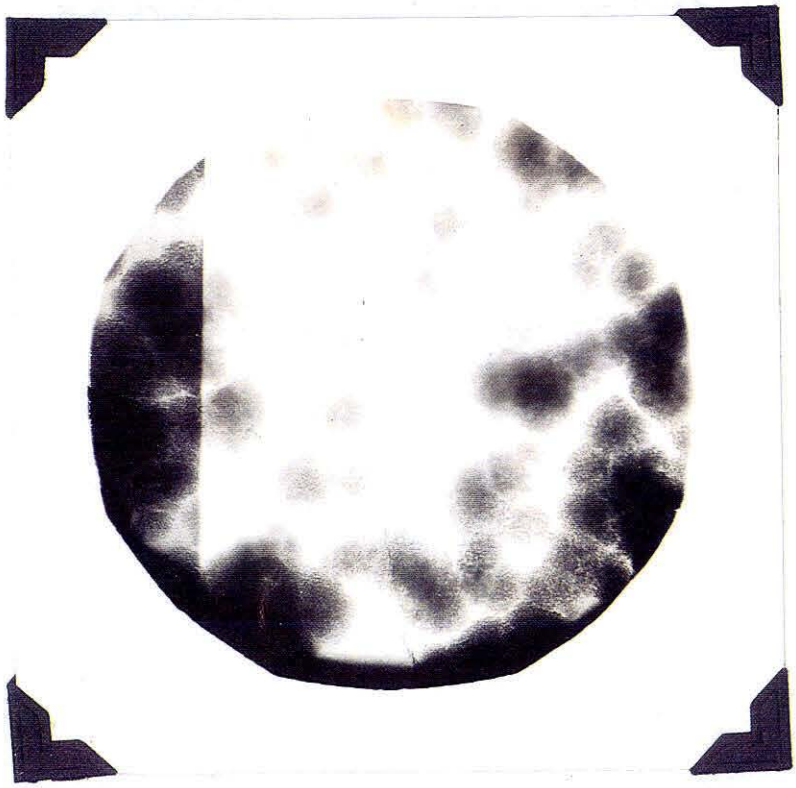
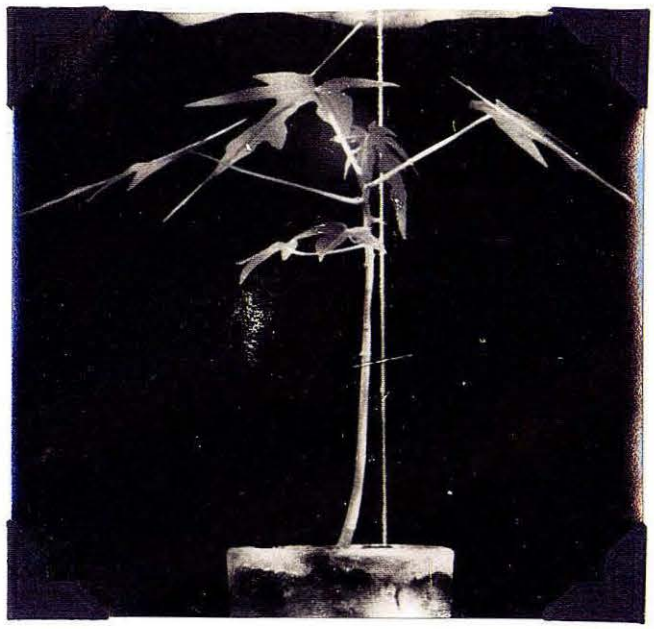


Plate V.

A. Day position of the leaves.



B. Night position of the leaves.