

STARCH CONVERSION IN LEAVES OF HELIANTHUS  
TUBEROSUS AND H. ANNUUS: PRELIMINARY  
OBSERVATIONS

BY

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1. INTRODUCTION

The knowledge of the biochemistry of carbohydrate conversion has strongly increased in the last decenniae (*cf.*, *e.g.*, 1). It seems that the development has been less strong in the field of the biology of carbohydrate conversion. In this statement it is understood that the study of the biochemistry of carbohydrate conversion is concerned with the pathways of these conversions and the enzymes involved. On the other hand, the study of the biology of carbohydrate conversion is concerned with the conditions under which these conversions take place in plant material.

The present paper is restricted to reporting some preliminary observations on starch conversion in leaves, especially in relation to leaf temperature.

The problem of starch conversion in leaves has several points of interest, a few of which may be mentioned. It can be looked upon as one of the major initial steps in the translocation of organic material, built up by photosynthesis. As such it probably counteracts some final steps of the photosynthetic chain (*i.e.*, the formation of starch) but, on the other hand, in the absence of any dissolution and translocation of starch, photosynthesis might come to a stand still. As to these questions, not much more than rather vague information exists. As will be demonstrated in the present paper — and has been known for a long time — leaves are depleted from starch more or less thoroughly in the dark, dependent on external conditions. It can be supposed that photosynthesis during the next day is more or less connected with the degree of depletion. During darkness, the process of starch conversion can be studied, apart from starch formation by photosynthesis. It may be supposed that starch conversion strongly influences other aspects of the nocturnal metabolism of a leaf. A more

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extensive knowledge of the underlying processes may be important also for the understanding of metabolic mechanisms connected with photoperiodic and thermoperiodic reactions. Starch conversion also may play a role in creating higher osmotic pressures, thus counteracting wilting. It may be mentioned here that, during the present investigation, the incidental observation was made that leaf fragments which, for some reason, had wilted during the experiment, as a rule showed a much stronger hydrolysis of starch than those that had remained turgescient.

The following reasons why our knowledge about the biology of starch conversion in leaves is not yet very complete, may be mentioned.

1. The variation and specialization as to plant species.
2. The rather complex operation required for complete sugar analysis.
3. The variation of individual leaves.

These points suggest the desirability of a simple method, allowing rapid, serial work.

## 2. MATERIAL AND METHODS

The experimental plants were *Helianthus tuberosus* (a clone, indicated by the name "Violet") and *H. annuus*, grown from seeds. Both were cultivated in the experimental field of this laboratory. Most experiments were made with halves of detached leaves, and with leaf discs. Some experiments were made with leaves attached to stem pieces of about 50 cm length, including the tip of the stem. In some cases, starch conversion was followed in leaves attached to the intact plants in the field. The work was begun in 1948; most of the experiments reported in this paper were made in the late summer and the early autumn of 1950. For the laboratory experiments, the leaves were collected at about sunset. The first series were made with leaf halves. The leaves were cut along the mid rib, the ribless half was used as a blank, and the half with the petiole served as experimental material. For this purpose, the petiole was introduced into a small bottle with water and placed under the conditions of the experiment. Six leaf halves were placed in each condition (*e.g.*, each temperature of a series). At the start of an experiment, the blanks were killed by quickly dipping them into boiling water three times in succession, and collected in glass boxes with 80 % ethanol for chlorophyll extraction. At the end of the experiment, the other leaf halves were treated likewise. The extraction of chlorophyll, including some renewals of ethanol, required about 3 days.

The starch determination was carried out in a very simple manner, briefly as follows. After the chlorophyll had been extracted (the leaves then being yellowish white), the starch was stained by replacing the ethanol by an iodine mixture according to SACHS (2). This mixture contained 10 g J in 1 l. 96 % ethanol, filled up to 2 l. with distilled water. Homogeneous staining was reached satisfactorily by staining the sets of leaf halves (*e.g.* 48 items) in large, glass covered glass boxes on a small shaking machine for some hours, or, eventually, a whole night. Evaporation of the iodine has to be prevented. The leaf halves remained in the staining mixture until the staining intensity was estimated. This estimation was made colorimetrically, using a photronic cell (mark "Megatron"), mounted in a square wooden frame of about 12 × 12 cm, and covered by an orange glass. The transmission curve of this glass was roughly similar to that of SCHOTT's OG 2. The amount of starch in a leaf half, treated as indicated above, was estimated by laying the leaf half as flatly as possible on the orange glass above the sensitive surface of the photronic cell.

The source of illumination was an incandescent bulb of 60 Watt at a distance of about 50 cm. The orange glass was intercalated, in order to increase the contrast between the blueish shade of the starch-rich leaf halves and the yellow color of those poor in starch, and also to decrease the straylight. The photo-electric current was measured with a  $\mu$ -amp meter; in the range used (up to 20  $\mu$ -amp) the deflection was linear in relation to the intensity of illumination of the photocell (fig.1.).

Mostly the measurements were made late in the evening. Then the constancy of the lamp was very good; it was checked many times, using a violet glass as a standard.

In the graphs, the  $\mu$ -amp values of the transmitted light are given. So, high values indicate little starch, low values much starch. The relation of the  $\mu$ -amp value to the starch content cannot be assumed to be linear, but a lower transmission will indicate a higher starch content and vice versa. This is easily seen by visual observation also. Considerations concerning BEER's law hardly seem worth while

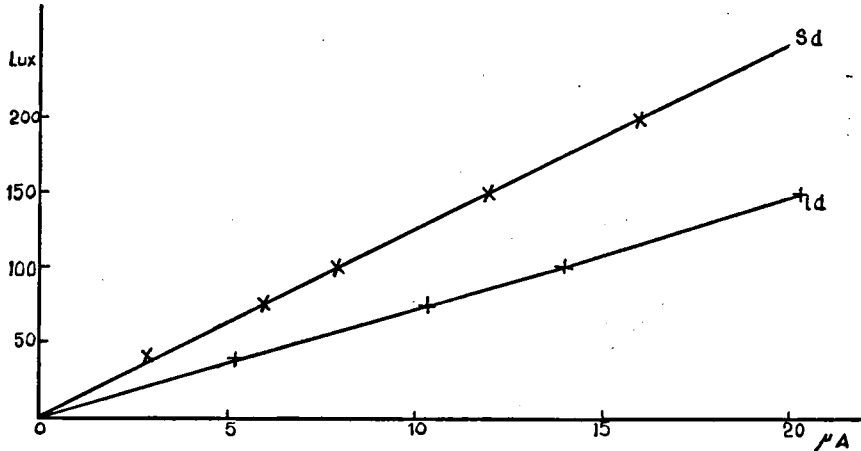


Fig. 1. Calibration of the light transmission meter. Lux values have been applied in special calibration cabinet, used for other calibrations in this laboratory also. sd = small, ld = large diaphragm. With orange glass (see text).

here, since the complicated behavior of the stray light in leaves with strongly different transmission values can hardly be estimated. But, for a preliminary survey, this does not seem necessary.

During the measurements, it was observed that as a rule the distribution of starch in a leaf half was very homogeneous. Therefore, in a further stage of the work, the exposition of half leaves was replaced by exposition of leaf discs of about 30 (in some cases about 25) mm in diameter, taken from the leaves in such a way that the larger ribs were avoided as much as possible. For a temperature series, a disc from each leaf was placed at each temperature; for an experiment 6 leaves were used. Thus, six discs were placed in a petri dish (one from each leaf), and such a dish was exposed at each temperature. The discs were mounted upon a gauze plate, resting upon glass bars with water underneath. At the bottom and inside the cover of the petri dish was moist filter paper.

Also these leaf discs, devoid of their connection with the midrib, showed extensive starch breakdown.

In this way a much more elegant set-up was obtained; an experiment with 6 replicates and 8-12 different conditions required only 6 leaves, and much smaller amounts of iodine than in the case of leaf halves, whereas they remained flat much better than half leaves. The simplicity of handling is especially demonstrated by the remark that, in 1950, in a period of about 6 weeks, near 5000 separate starch estimations have been made.

### 3. EXPERIMENTAL RESULTS AND THEIR DISCUSSION

#### a. The course of the starch content of leaves during the daily cycle

Fig. 2 shows observations on the course of the starch content during the daily cycle in leaves of *Helianthus tuberosus* under natural conditions, on October 4, 5 and 6, 1950. The observations were made by taking



in the afternoon (between 16 and 18 h). It should be noted that the shape of the curves cannot be evaluated as depletion rate curves or starch formation rate curves, since the numerical relationship between starch content and light transmission is not known. Nevertheless, some curves suggest that starch dissolution in the second part of the night is more effective than in the first few hours of darkness. Table 1 gives some atmospheric data on the days of the observations.

TABLE 1  
Some atmospheric data belonging to the observations on starch conversion in leaves of *Helianthus tuberosus* under natural conditions. Exp. of 4-6 October 1950.

Date	Hour	Temp. (°C)	Remarks
1950 4.10	7.30	10.2	—
	11.30	14.8	covered
	13.30	15.6	covered
	15.30	15.9	covered
5.10	6.—	8.5	lightly covered
	9.—	10.0	lightly covered, sun coming through
	11.15	15.0	lightly covered, weak sun
	13.15	18.1	sunny, somewhat hazy
	15.—	18.8	sunny
	17.30	16.0	no sun on the plants any more
6.10	24.—	11.5	clear
	6.—	10.0	clear with light fog
	9.—	13.2	sunny
	11.45	19.0	sunny
	13.45	21.1	sunny
	16.45	19.8	sunny. Only 4 leaves left. Points not in graph.

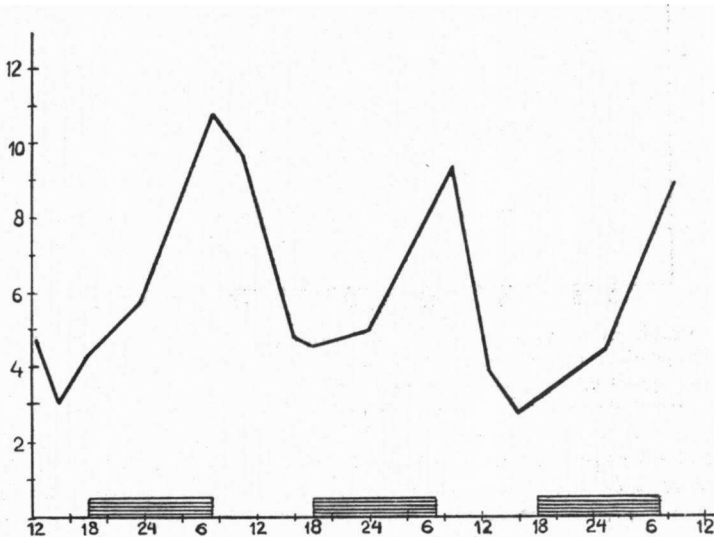


Fig. 3. Same as fig. 2, for *Helianthus annuus*. Average of 4 leaves. Exp. of 13, 14, 15 and 16 October 1950.

Fig. 3 shows a similar curve, taken on October 14–16, 1950 in healthy leaves of *Helianthus annuus*. This curve is an average of 4 leaves. The behavior is the same, even in details, as observed in *H. tuberosus*.

Table 2 gives atmospheric data pertaining to the observations in *H. annuus*. In relation to this experiment, light intensities have been measured along with the collection of leaf samples, with the aid of the spherical radiation meter (*cf.* 3).

The curves of fig. 2 and 3 do not contain any suggestion that the process of starch metabolism shows conspicuous differences in a short day plant (the race of *H. tuberosus* used) as compared with a plant flowering in long days (*H. annuus*). More observations, however, are needed to judge whether this applies more generally.

#### *b. Starch hydrolysis in relation to temperature*

Most of the experiments reported in this paper are concerned with the influence of temperature. The samples were exposed in darkness in thermostates of the temperatures indicated. Either leaf halves with their petioles in small bottles with water, or discs, mounted on gauze in petri dishes (*cf.* section 2) were used. Mostly 8 temperatures, from 3° to 31° C, sometimes 12 temperatures, between — 3° and 40° C, were used.

Some characteristic curves are shown in figs 4, 5, 6, 7 and 8. Fig. 4

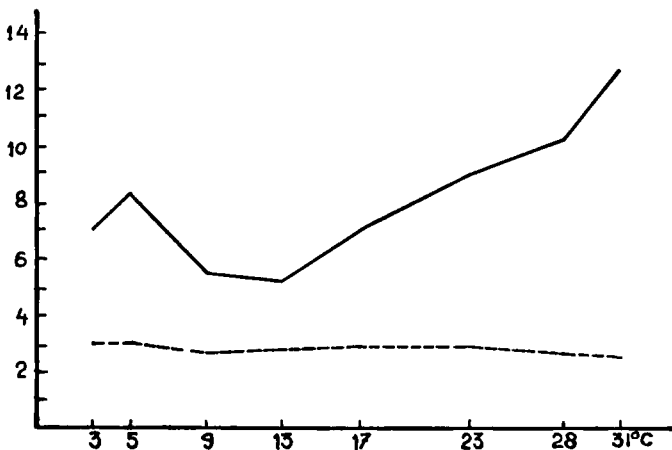


Fig. 4. Starch hydrolysis in relation to temperature. Leaf halves of *Helianthus tuberosus*. Exposition time 6½ hours. Dotted line: Control at the start of experiment. Full drawn line: Light transmission (ordinate) at the end of the experiment. Each point average of 6 leaves. Exp. of 13–14 September 1950.

represents an experiment of relatively short duration (6½ h) with leaf halves of *H. tuberosus*; fig. 5 shows a similar experiment with leaf discs (∞ 30 mm diam. ) of the same species (duration 8½ h) over a more extensive temperature range. Fig. 6 shows a similar experiment, made with smaller leaf discs and over a longer experimental period (14½ h).

TABLE 2  
 Some atmospheric data pertaining to the experiment on starch conversion in leaves of *Helianthus annuus* under natural conditions.  
 Light intensities in  $\mu$ -Watts/cm<sup>2</sup> cross section, measured with the spherical radiation meter (3). Exp. of 13-16 October 1950

Date	13.10.1950				14.10.1950				15.10.1950			16.10.1950
	Hour	12.15	14.30	17.30	7.30	10.45	13.15	16.00	9.15	13	16.00	8.45
Leaf number												
1	Light Intensity	∞ 60000	22600	1240	5200	14500	16000	12000	15000	38000	13000	3800
2		∞ 60000	8400*	550	2400	11300	10200	7800	5900	18000	∞ 9000	2700
4		∞ 14500	∞ 60000	285	3250	16200	∞ 15000	8700	9700	24000	10200	3150
5		∞ 60000	∞ 60000	260	3050	16700	∞ 15000	9500	9700	16000	10500	3350
Temp. (° C)		19	20.5	13	8	14	14	12	9	14	14	10.5
Atmospheric condition		bright sun the whole day			cloudy	cloudy	slightly cloudy	slightly cloudy	foggy	slightly clouded	no direct sun	cloudy

\* leaf shaded

Fig. 7 shows a short duration experiment over a large temperature range with large discs of *H. annuus*; fig. 8 shows a similar experiment with smaller discs over a longer period. In all cases 6 items were averaged for each point.

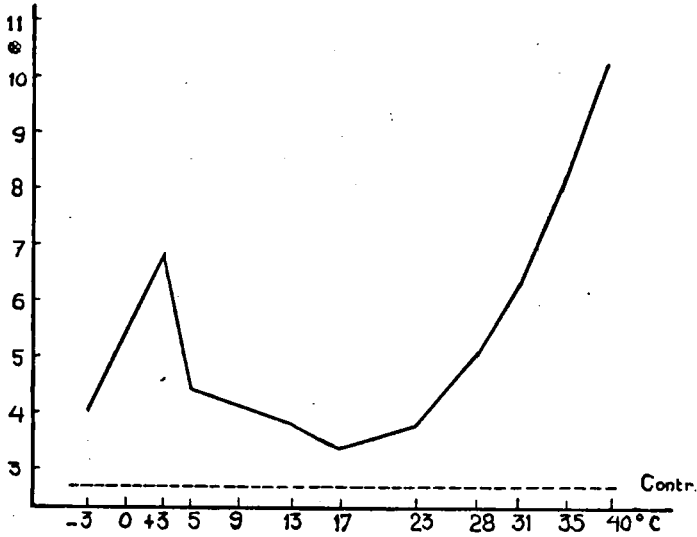


Fig. 5. Starch hydrolysis in relation to temperature. Leaf discs from *Helianthus tuberosus*. Exposition time 6½ hours. Dotted line: control at the start (horizontal line through the average ordinate of 6 discs). Full drawn line: light transmission (ordinate) at the end of experiment. Control, and hydrolysis value at each temperature average of 6 discs, derived from the same set of 6 leaves for all temperatures. Exp. of 9-10 October 1950.

The following features of the curves are obvious. In practically all cases (with the exception of the short duration experiment with *H. annuus* (fig. 7)), starch hydrolysis in relation to temperature shows two maxima separated by a, mostly very pronounced, minimum. In both species the first maximum develops between  $-3^{\circ}$  and  $+5^{\circ}$  C, with the highest values probably between  $0^{\circ}$  (or somewhat below  $0^{\circ}$ ) and  $+3^{\circ}$  C. This obviously coincides with the region in which potato tubers turn "sweet". So far, however, I have not attempted a direct comparison of both reactions. Also, the tubers of *Helianthus tuberosus* have not been investigated in this respect, so far. It should be noted that the reaction in the leaves is the same in *H. tuberosus* and *H. annuus*, the latter not forming tubers. The minimum in the hydrolysis curve extends from about  $5^{\circ}$  to  $20^{\circ}$  C, the deepest region being situated between  $9^{\circ}$  and about  $15^{\circ}$ . From about  $15^{\circ}$  onward the hydrolysis curve shows a rather rapid increase. In the experiments of short duration, the degree of hydrolysis increases up to the highest temperatures, applied in both species (fig. 5, 7). In the experiments of longer duration, hydrolysis shows about the same (high) values from about  $28^{\circ}$  onward, probably meaning complete hydrolysis of the starch present. In fig. 8, the  $40^{\circ}$  C



point is abnormally low; in this case all 6 discs had a blackish necrotic appearance at the end of the experiment. Especially at 17° C, the hydrolysis value, with respect to the minimum values recorded at slightly lower temperatures, is rather sensitive to the duration of the experiment. It should be remarked especially that even at -3° C

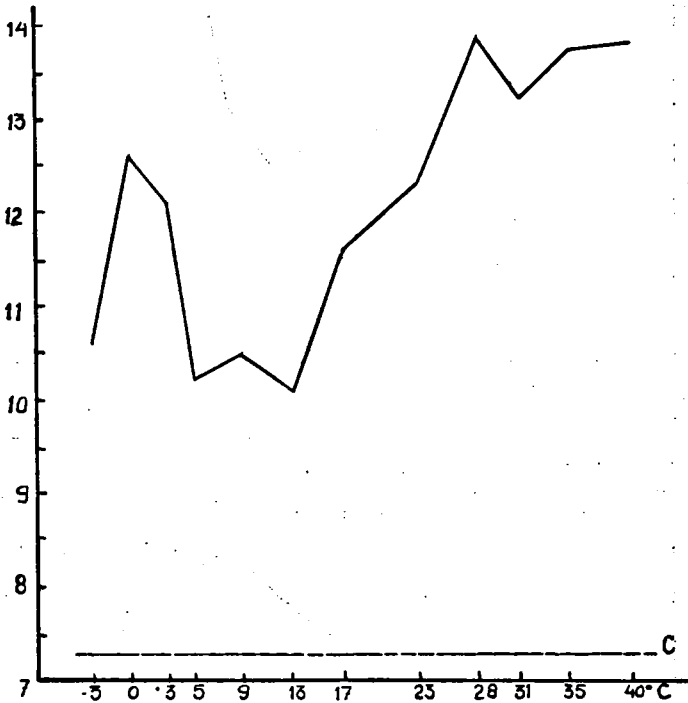


Fig. 6. Starch hydrolysis in extensive temperature range. Leaf discs of *Helianthus tuberosus*. Exposition time 14½ h., 6 leaves. See further: caption of fig. 5. Exp. of 29-30 September 1950.

an appreciable hydrolysis takes place (*cf.*, the corresponding blanks, taken prior to the experimental period from the same leaves). Its value is more or less comparable to that at about +13° C.

Some considerations concerning the possible nature of the minimum will be given below.

A further remark to be made is that hydrolysis, as to amount and temperature characteristics, does not show any conspicuous differences whether leaf halves or discs are used. It will be shown presently, that also the connection of the leaves to a stem piece does not make any difference. In all cases hydrolysis can run fairly to completeness in the duration of one night, provided the other conditions are favorable. It seems possible to conclude (provisionally) that in first instance starch hydrolysis is a cellular process which may proceed to completeness irrespective of transport possibilities over long distances.

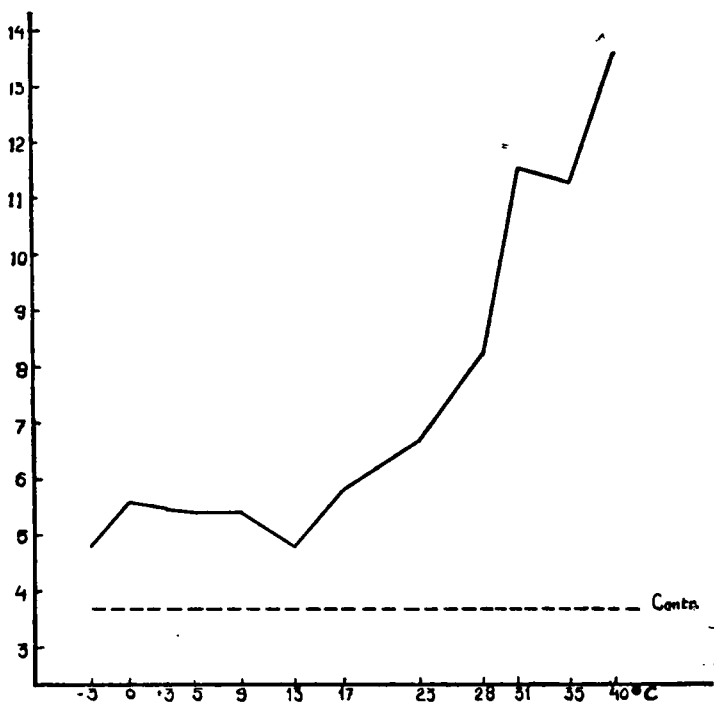


Fig. 7. Starch hydrolysis in extensive temperature range. Leaf discs from *H. annuus*. Exposition time  $8\frac{1}{2}$  h., 6 leaves. See further: caption of fig. 5. Exp. of 9-10 October 1950.

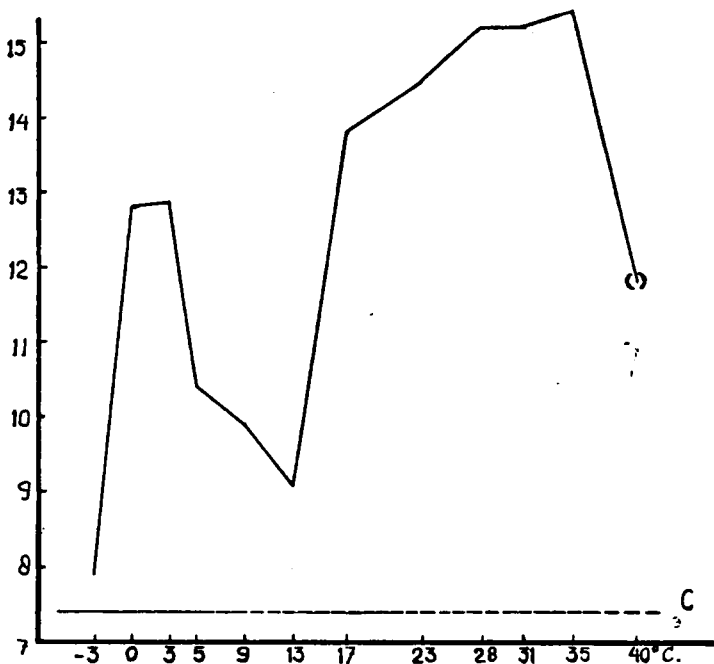


Fig. 8. Starch hydrolysis in extensive temperature range. Leaf discs from *H. annuus*. Exposition time  $14\frac{1}{2}$  h., 6 leaves. See further: caption of fig. 5. Exp. of 29-30 September 1950.

Fig. 9 represents the experiment, already alluded to, in which starch hydrolysis in relation to temperature was investigated in leaves connected with a large stem piece. The set-up was as follows. Three stems (each including the stem tip) were placed in a large bottle with water, in each temperature. The three stems belonged to three different plants, A, B, C, but all A's (in the various temperatures) were of the same plant. So were all B's and all C's. The temperatures

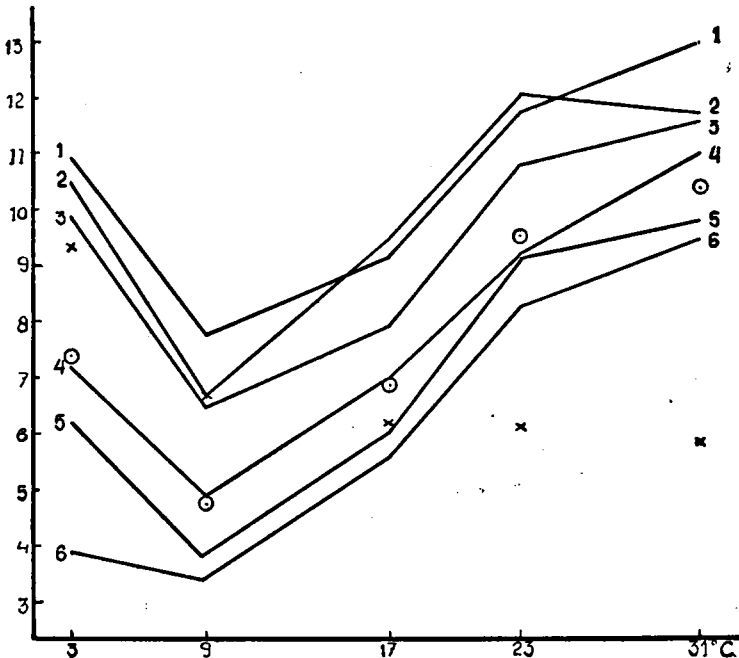


Fig. 9. Starch hydrolysis in relation to temperature. Discs from leaves of *H. tuberosus* attached to large stem pieces. 1-6 increasing age of leaves. Each point average of 3 sprouts. Ordinates: Difference in light transmission values at the end of the experiment as compared with the controls at the start. Curves 1-6 average of 3 (in some cases 2) values. See Table 3. For the sake of clarity, the ordinate of curve 1 has been increased by 2 units, that of curves 2 and 3 by 1 unit. ○ average of curves 1-6, × distance between curves 1 and 6 (as plotted) in order to illustrate the special difference at 3° C. Exp. of 4-5 October 1950.

chosen were 3°, 9°, 17°, 23° and 31° C. A disc was cut out of each of 6 leaves from each stem at the start of the experiment, as follows. About three uppermost visible leaves were left untouched, these being too small. The next leaf was the leaf No. 1 (fig. 9); leaf No. 2 was 2 leaves down from 1, No. 3 was 2 leaves down from 2, etc. Under No. 6, the stem pieces still had 4 leaves, and below that, a defoliated stem piece of about 12 cm was left. A corresponding disc from every leaf used was cut out at the end of the experiment. The experimental period was about 15 hours.

Each point of the curves, obviously, is the average of 3 values

TABLE 3

Data on starch hydrolysis in *Helianthus tuberosus* (expressed as light transmission values) in relation to temperature in leaves of various ages, attached to large stem pieces. The data "Experiment minus control" are plotted in fig. 9. C = control. Exp. of 4-5 October 1950.

Plant	Leaf	3°		9°		17°		23°		31°	
		C	Exp.	C	Exp.	C	Exp.	C	Exp.	C	Exp.
A	1	5.4	13.4	5.5	8.7	2.2	4.2	5.0	13.6	2.5	12.0
B	1	4.5	15.0	2.3	9.5	4.0	14.0	2.7	13.3	1.8	14.0
C	1	4.0	12.0	4.2	11.0	5.6	14.7	3.3	13.3	1.3	12.8
Sum . . . . .		13.9	40.4	12.0	29.2	11.8	32.9	11.0	40.2	5.6	38.8
Average . . . . .		4.6	13.5	4.0	9.7	3.9	11.0	3.7	13.4	1.9	12.9
Exp. minus control		8.9		5.7		7.1		9.7		11.0	
A	2	5.3	14.3	5.5	10.0	3.0	9.0	6.0	14.8	5.2	14.0
B	2	4.5	15.0	3.2	10.0	4.0	13.2	2.5	15.6	1.8	13.0
C	2	4.0	13.0	—	—	4.0	14.3	4.0	16.0	1.7	13.8
Sum . . . . .		13.8	42.3	8.7	20.0	11.0	36.5	12.5	46.4	8.7	40.8
Average . . . . .		4.6	14.1	4.4	10.0	3.7	12.2	4.2	15.3	2.9	13.6
Exp. minus control		9.5		5.6		8.5		11.1		10.7	
A	3	5.1	12.4	7.2	10.5	5.0	9.1	4.2	12.4	4.8	12.3
B	3	4.0	14.5	—	—	5.3	15.0	—	—	3.0	14.5
C	3	—	—	7.0	14.6	—	—	6.3	17.8	1.6	14.3
Sum . . . . .		9.1	26.9	14.2	25.1	10.3	24.1	10.5	30.2	9.4	41.1
Average . . . . .		4.6	13.5	7.1	12.6	5.2	12.1	5.3	15.1	3.1	13.7
Exp. minus control		8.9		5.5		6.9		9.8		10.6	
A	4	7.0	11.8	5.0	8.0	4.7	8.0	4.7	13.3	2.2	10.3
B	4	3.2	12.0	5.0	10.3	5.3	12.7	2.4	12.3	3.0	15.6
C	4	4.0	12.0	3.2	10.7	3.0	13.2	—	—	1.4	13.7
Sum . . . . .		14.2	35.8	13.2	28.0	13.0	33.9	7.1	25.6	6.6	39.6
Average . . . . .		4.7	11.9	4.4	9.3	4.3	11.3	3.6	12.8	2.2	13.2
Exp. minus control		7.2		4.9		7.0		9.2		11.0	
A	5	4.8	10.0	2.0	6.2	3.0	5.0	4.3	12.4	2.4	9.6
B	5	4.3	12.0	7.0	9.8	6.5	14.3	2.8	12.5	4.6	15.8
C	5	2.2	8.0	9.0	13.4	3.0	12.0	3.3	13.0	2.0	13.1
Sum . . . . .		11.3	30.0	18.0	29.4	12.5	31.3	10.4	37.9	9.0	38.5
Average . . . . .		3.8	10.0	6.0	9.8	4.2	10.4	3.5	12.6	3.0	12.8
Exp. minus control		6.2		3.8		6.2		9.1		9.8	
A	6	8.0	9.8	4.0	7.3	2.5	6.0	6.0	12.8	3.8	10.3
B	6	4.5	9.2	7.7	7.6	6.0	12.0	6.2	15.0	3.0	13.4
C	6	3.7	8.8	7.6	14.4	3.3	10.5	2.3	11.4	2.0	13.4
Sum . . . . .		16.2	27.8	19.3	29.3	11.8	28.5	14.5	39.2	8.8	37.1
Average . . . . .		5.4	9.3	6.4	9.8	3.9	9.5	4.8	13.1	2.9	12.4
Exp. minus control		3.9		3.4		5.6		8.3		9.5	
Average of "Exp. minus control" leaves 1-6 . . .		7.4		4.8		6.9		9.5		10.4	

(obtained from the corresponding leaf number of the 3 stems, A, B, and C). The corresponding blank also is the average of 3 values, obtained in a similar way. The difference of these averages is plotted in fig. 9 (see also Table 3).

The following conclusions can be drawn: 1. The curves do not differ conspicuously from those obtained with cut leaves or leaf discs. 2. Again, relatively high values at 3° and at 17° and higher are separated by a clear minimum at 9° C. 3. Leaves of all ages investigated behave in a virtually similar way. 4. There is a slight indication that the older leaves show a less conspicuous maximum of hydrolysis at low temperatures.

In some other experiments (figs 10, 11) leaves were brought successively under various temperatures. These experiments were set

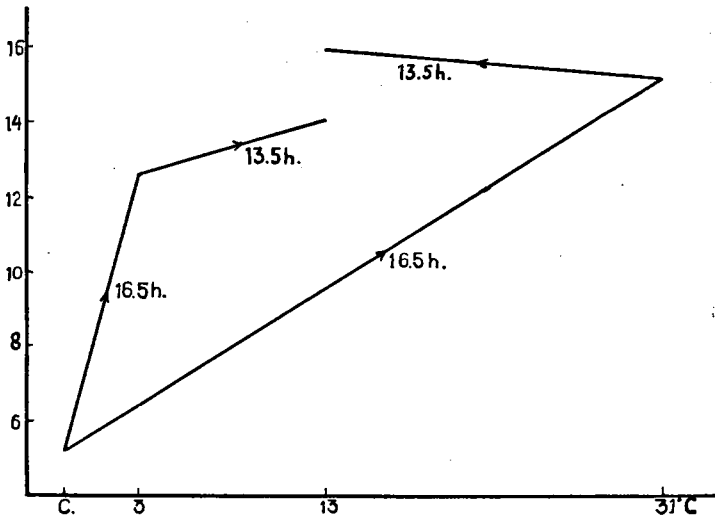


Fig. 10. Starch hydrolysis at 13° C in leaf discs of *Helianthus tuberosus*, after pre-exposure to 3° C and 31° C respectively. Ordinate at C = light transmission at the start. Duration of pre-exposures 16.5 h., of exposure to 13° C: 13.5 h. Ordinate: light transmission. Each point average of 6 leaf discs. Exp. of 9-10 October 1950.

up in order to attempt an elucidation of the minimum in the hydrolysis temperature curve. It must be mentioned first that, so far, no rates of partial processes have been measured along with the experiments. It would seem that the observed temperature curve might be explained by assuming three different processes, viz., 1. hydrolysis of starch, 2. synthesis (or resynthesis) of starch, 3. respiration of sugars. It seems feasible to assume that these processes all have a positive temperature increment, but of different values, somewhat like schematically represented in fig. 12. According to this scheme, hydrolysis would develop the largest potential velocity of the three at low temperatures, and, since it does not rely upon the velocities of the two other ones, hydrolysis will actually predominate. In this temperature region,

sugars will accumulate. At average temperature, however, (re)-synthesis will acquire a rate commensurable to that of hydrolysis, thus counterbalancing hydrolysis to a large extent, and decreasing its observed gross rate. At still higher temperatures the potential rate of respiration will be the highest of the three, thus preventing re-synthesis by the removal of sugars produced by starch hydrolysis.

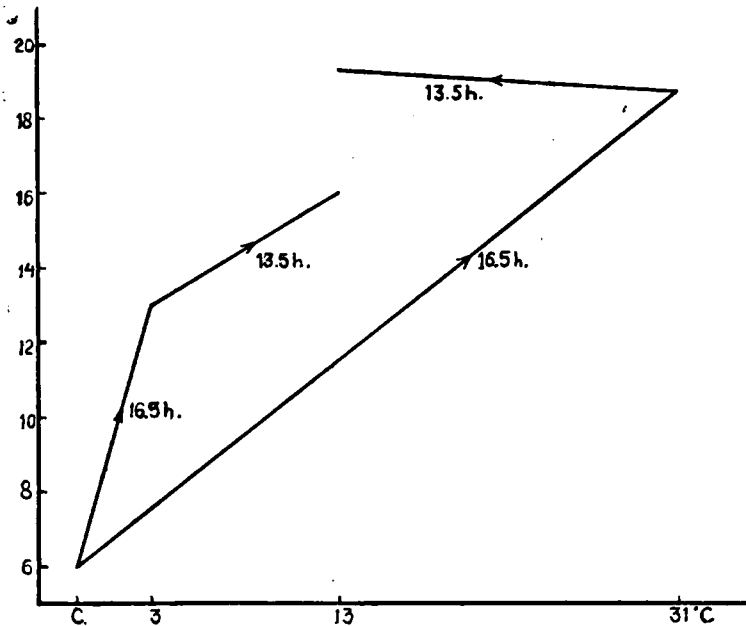


Fig. 11. The same as fig. 10, with *H. annuus*. Exp. of 9-10 October 1950.

(Actually, since respiration may be assumed to be limited by the amount of hydrolytic material formed, the curve of starch disappearance will again follow the hydrolysis curve). Two different situations have been represented in fig. 12, differing in the position of the synthesis curve. The position I would seem to imply that at medium temperatures hydrolysis would be completely prevented by the synthetic tendency, in the case II it would only be reduced in its gross rate. The experiments presented so far are in favour of case II (see below).

The experiments of figs 10 and 11 were made to investigate this point more directly. The idea was that, probably, a prolonged exposure to low temperature might increase the sugar content so much that, after transfer to about 13°, resynthesis of starch might be demonstrated. Under these conditions, pre-exposure at high temperature, would not be expected to lead to synthesis, since the sugars should be removed by respiration.

The experiments were made in such a way that discs from the same leaf were brought in part to 3° C in part to 31° C, and left there for

16.5 h. After that, some were examined for starch hydrolysis having taken place, others were transferred to 13° C, and left there for another 13.5 h. Figs 10 and 11 show that both in *Helianthus annuus* and in *H. tuberosus* hydrolysis increases during the period at 13° C. It even may be remarked that hydrolysis at 13° C proceeds more strongly after pre-exposure to 3° than it does after pre-exposure to 31° C, especially in the case of *H. annuus*. It should not be overlooked, however, that the pre-exposure at 31° C had removed more starch already

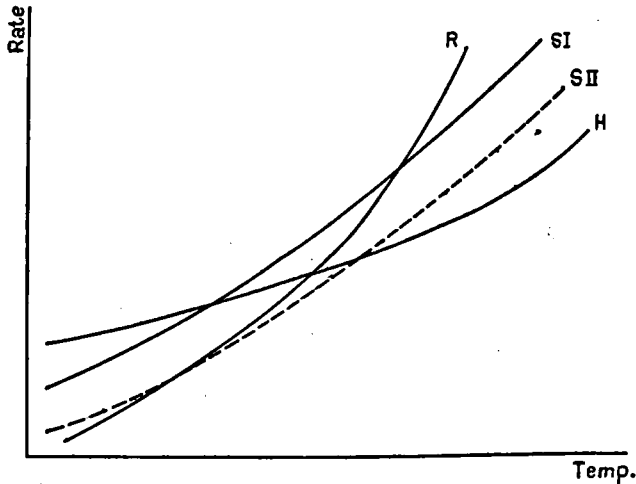


Fig. 12. Scheme representing possible increments of starch hydrolysis (H), (re)-synthesis of starch (SI and SII respectively) and respiration (R) in *Helianthus* leaves, in order to explain the minimum in the temperature-hydrolysis curve. See text.

then the pre-exposure at 3° C, so that no big further increase of the light transmission was to be expected. But the fact remains, that after preexposure to 3° C, no resynthesis of starch at 13° C can be demonstrated. The explanation may be that the situation corresponds to that of case II in fig. 12. On the other hand, resynthesis may obtain only at high local sugar concentrations, concurrent with hydrolysis in the neighbourhood of starch particles at suitable temperatures, and does not take place when the concentration of the sugar is decreased by diffusion.

*c. Starch hydrolysis in relation to some other factors*

A number of experiments has been made in order to investigate the influence of light on starch hydrolysis, the influence of the gas-phase, especially the oxygen content, and of the CO<sub>2</sub> content in relation to light. Also the admission of glucose and saccharose in various ways has been studied. Not all results obtained along these lines are as satisfactory yet as those obtained in the temperature studies, since certain experimental difficulties have not yet been fully eliminated. The following results seem to be worth mentioning.

Figs 13 and 14 show an experiment in which the influence of light of various intensities, including darkness has been studied at different temperatures. Daylight fluorescent tubes have been used for illumination. Six discs were put in petri dishes above 0.5 % KOH solution, the No. 1 disc in each petri dish coming from the same leaf, each No. 2 disc from a second leaf, and so on. The leaf material exposed in the various conditions (16 experiments and one control) thus is strictly comparable. No definite effect, consistent at all temperatures, could be traced. The only conclusion seems that under the conditions chosen (low carbon dioxide) no definite effect of illumination on

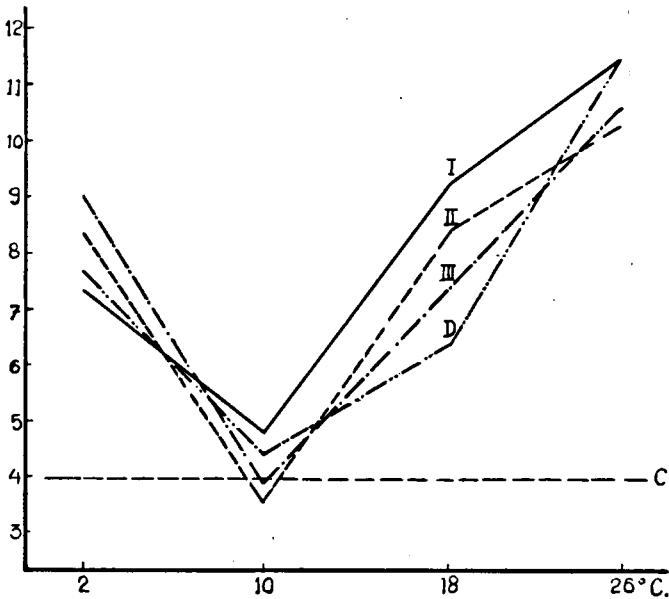


Fig. 13. Starch hydrolysis in relation to temperature at various light intensities. Leaf discs of *H. tuberosus*. Low  $\text{CO}_2$ -concentration. Light intensities at 2° C: I  $\approx$  2600 lux, II  $\approx$  1300 lux, III  $\approx$  650 lux; at 10° C: I  $\approx$  2600 lux, II  $\approx$  2100 lux, III  $\approx$  1200 lux; at 18° C: I  $\approx$  3400 lux, II  $\approx$  1900 lux, III  $\approx$  900 lux; at 26° C: I  $\approx$  5000 lux, II  $\approx$  2100 lux, III  $\approx$  900 lux; D = dark. See further caption of fig. 5. Exp. of 11-12 October 1950.

starch hydrolysis has been found, neither in *H. tuberosus*, nor in *H. annuus*. All curves show the characteristic temperature dependence, as described earlier in this paper, with a minimum at 10° C. This minimum is more pronounced in *H. tuberosus* than in *H. annuus*, which is also in accordance with what was mentioned above.

In 1951, some series of experiments with *H. tuberosus* were made in order to investigate the influence of  $\text{CO}_2$ , in the presence and absence of light. Leaf discs were exposed in glass jars (so called dissiicators were used) which were filled either with normal air or with air enriched with 5 %  $\text{CO}_2$ . The results are given in Table 4. Light +  $\text{CO}_2$  definitely decreases hydrolysis as compared with darkness +  $\text{CO}_2$  or



with light + air. This seems to indicate, that photosynthesis counteracts starch hydrolysis in the presence of CO<sub>2</sub>. This time, in air, also a difference between light and darkness is observed. But also in darkness, hydrolysis is definitely less in the presence of 5 % CO<sub>2</sub>, which

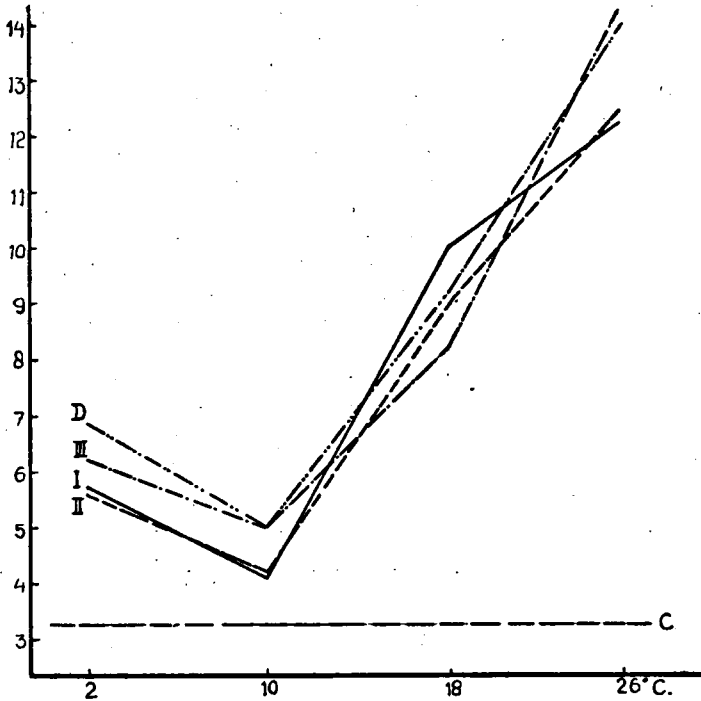


Fig. 14. The same experiment as fig. 13, with *H. annuus*. Exp. of 11–12 October 1950.

TABLE 4  
Influence of light and darkness on starch hydrolysis in leaf discs of *Helianthus tuberosus* with and without CO<sub>2</sub> added.

Date	Air + 5 % CO <sub>2</sub> , light	Air + 5 % CO <sub>2</sub> , darkness	Air, light	Air, darkness	Control
1951					
30/8–31/8 . . . . .	2.7	2.8	4.6	5.5	1.2
31/8– 1/9 . . . . .	2.3	2.0	2.4	3.3	0.6
5/9– 6/9 . . . . .	3.8	8.3	7.1	9.4	1.6
12/9–13/9 . . . . .	2.0 *	8.2 *	5.3	9.6	1.0
13/9–14/9 . . . . .	1.6	3.8	3.0	6.6	1.0
14/9–15/9 . . . . .	1.2	5.6	4.9	7.1	1.3
Average . . . . .	2.3	5.1	4.6	6.9	1.1
Average II . . . . .	(2.3)	4.5	4.4	6.4	(1.1)

\* These values were found in the reverse order as indicated here. It seems certain that both vessels with the leaf discs in iodine solution have been interchanged. Omitting this experiment, the average values of the remaining 5 experiments are as indicated in the last horizontal line (Average II).

seems to point to some harmful effect of the  $\text{CO}_2$ -tension used. The experiments were made at about  $25^\circ\text{C}$ .

Already in 1950, it has been tried to study the effect of sugars on starch hydrolysis, by observing leaf discs in glucose or sucrose solutions of low depth, or a shaking machine. The results had not been very satisfactory. In some new attempts leaf discs were infiltrated with sugar solutions in vacuo, dried superficially afterwards, and then submitted to conditions for starch hydrolysis. In all cases the infiltration with various strengths of sugar solution counteracted hydrolysis, but so did also infiltration with water. This result was interpreted to mean that removal of air from the intercellular spaces probably interfered with various aspects of the metabolism of the leaf.

This led to experiments in which hydrolysis of starch in leaf discs was investigated in a gasphase of nitrogen containing different amounts of oxygen. In most of the experiments, 6 leaf discs were placed in tubes of about 100 ml. contents, with a rubber stopper having in- and outlet for gas. All experiments were made in darkness at  $5^\circ$  and  $25^\circ\text{C}$ . Some of the results are collected in Tables 5 and 6. It was observed that leaf discs, exposed in nitrogen, showed black spots at the end of the experiment. It was thought that a more or less quantitative idea of the damage done could be obtained by

TABLE 5

Transmission of leaf discs of *Helianthus tuberosus* before iodine staining, in connection with the oxygen content of the gas phase (see text). Exps. of various dates.

Date	$25^\circ\text{C}$		Control	$5^\circ\text{C}$		Remarks
	Air	$\text{N}_2$		Air	$\text{N}_2$	
1951						
17/9-18/9	34.5	32.7	31.6	35.0	22.3	Pure nitrogen
18/9-19/9	35.8	6.1	32.3	36.6	33.1	" "
19/9-20/9	38.0	24.6	34.4	37.4	35.8	" "
20/9-21/9	35.9	7.7	30.0	35.2	34.2	" "
21/9-22/9	35.1	7.8	33.4	34.4	31.4	" "
Average	35.9	15.8	32.5	35.7	31.4	
25/9-26/9	33.5	31.5	25.7	35.1	32.4	$\text{N}_2$ , containing 3.83 % $\text{O}_2$
26/9-27/9	29.7	16.4	28.7	28.7	27.3	" " " "
27/9-28/9	28.4	17.9	21.0	26.5	21.7	" " " "
Average	30.5	21.9	25.1	30.1	27.1	
1/10-2/10	32.8	28.6	27.5	31.7	31.3	$\text{N}_2$ , containing 5 % $\text{O}_2$
2/10-3/10	33.5	24.3	29.7	33.0	31.2	" " " "
3/10-4/10	32.1	29.3	29.6	32.1	31.9	" " " "
4/10-5/10	32.1	28.8	29.3	32.4	30.1	" " " "
Average	32.6	27.8	29.0	32.3	31.1	
5/10-6/10	30.6	26.1	26.4	29.8	28.1	$\text{N}_2$ , containing 10 % $\text{O}_2$
8/10-9/10	30.0	29.2	26.8	28.7	28.8	" " " "
Average	30.3	27.7	26.6	29.3	28.5	

measuring the light transmission of the discs before colouring with iodine solution. These data are collected in Table 5. It is seen that, in pure nitrogen, mostly the light transmission is strongly decreased at 25° C, owing to the dark spots. These dark spots obviously arise through the action of oxidases, set free by the disorganization of certain cell complexes. Also at an oxygen tension of 3.83 %, this influence still is evident, and some slight remainders of it are seen at 5 and 10 % O<sub>2</sub>. At 5° C, this effect is only very slightly, if at all perceptible. Only in pure nitrogen the average transmission value of the leaf discs is slightly lower than that of the controls. In air, the transmission values show no differences at 5° and 25° C. They are even higher than those of the controls. This last mentioned fact is

TABLE 6

Transmission of iodine-stained leaf discs of *Helianthus tuberosus* (see text) in connection with the oxygen content of the gas phase. Exps. of various dates.

Date	25° C		Control	5° C		Remarks
	Air	N <sub>2</sub>		Air	N <sub>2</sub>	
1951						
17/9-18/9	10.5	4.7	2.4	13.1	4.0	Pure nitrogen
18/9-19/9	10.9	2.6	3.2	12.9	9.3	" "
19/9-20/9	14.6	11.0	2.5	13.4	5.2	" "
20/9-21/9	10.3	2.1	1.7	12.1	3.9	" "
21/9-22/9	7.4	1.8	2.0	11.0	3.3	" "
Average	10.7	4.4	2.4	12.5	5.1	
25/9-26/9	8.5	7.3	1.4	13.0	5.2	N <sub>2</sub> , containing 3.83 % O <sub>2</sub>
26/9-27/9	7.9	8.7	2.0	11.9	7.0	" " " "
27/9-28/9	10.1	6.4	1.6	11.5	5.1	" " " "
Average	8.8	7.5	1.7	12.1	5.5	
1/10-2/10	10.1	7.7	1.6	9.7	4.9	N <sub>2</sub> , containing 5 % O <sub>2</sub>
2/10-3/10	12.8	8.9	2.0	11.4	7.4	" " " "
3/10-4/10	5.4	4.0	1.3	9.5	4.0	" " " "
4/10-5/10	10.2	9.2	1.9	13.5	8.0	" " " "
Average	9.6	7.5	1.7	11.0	6.1	
5/10-6/10	6.5	3.0	1.5	9.0	5.0	N <sub>2</sub> , containing 10 % O <sub>2</sub>
8/10-9/10	6.4	4.3	1.9	9.0	4.3	" " " "
Average	6.5	3.7	1.7	9.0	4.7	

not well understood; the explanation might be that the larger amount of starch, present in the control, decreases the light transmission value by back scattering of light. At 5°, in nitrogen with 5 or 10 % O<sub>2</sub> the transmission values are not sensibly different from those in air, thus, no damage is perceivable in this way.

Looking at Table 6, in which the light transmission of the iodine stained leaf discs are given, we see that in all cases, starch hydrolysis in nitrogen is much less than in air, also at 5° C and even with 10 %

oxygen. It is remarkable that, invariably, hydrolysis in air was found more extensive at 5° than at 25° C. This seems slightly different from the experience obtained in the temperature series. It may be connected with the small air space available for the leaf discs, and it may be due to a relative lack of oxygen, or to some harmful excretion of the leaf discs into the atmosphere. The difference between air and the various low oxygen tension gas phases shows, that both at 5° and 25° C, oxygen promotes starch hydrolysis. Whether this influence is a direct one on the process of starch hydrolysis *sensu stricto*, or an indirect one via the metabolism of the leaf disc as a whole, cannot be inferred from the present experiments. The latter possibility seems the more likely one.

TABLE 7

Transmission of leaf discs of *Helianthus tuberosus* in connection with the oxygen content of the gas phase (see text). Experiments in disiccators. Exps. of various dates.

Date	25° C		Control	5° C	
	Air	N <sub>2</sub>		Air	N <sub>2</sub>
A. Prior to iodine staining					
1951					
15/10-16/10 . . . . .	30.8	27.2	27.7	28.3	26.6
16/10-17/10 . . . . .	26.5	27.8	18.1	27.9	28.6
17/10-18/10 . . . . .	28.4	24.6	27.1	29.0	29.1
18/10-19/10 . . . . .	29.3	25.3	25.1	25.9	27.4
19/10-20/10 . . . . .	27.1	22.3	24.1	27.4	26.1
23/10-24/10 . . . . .	30.5	24.5	27.4	31.1	27.1
24/10-25/10 . . . . .	30.3	28.1	24.6	29.7	27.9
25/10-26/10 . . . . .	30.4	24.3	26.9	28.5	28.8
Average . . . . .	29.2	25.5	25.1	28.5	27.7
B. After iodine staining					
15/10-16/10 . . . . .	20.7	15.0	7.2	19.4	17.0
16/10-17/10 . . . . .	16.3	14.2	—	19.7	18.4
17/10-18/10 . . . . .	—	20.8	11.6	21.7	21.6
18/10-19/10 . . . . .	20.3	—	8.2	19.9	17.2
19/10-20/10 . . . . .	19.1	17.3	6.3	18.6	—
23/10-24/10 . . . . .	—	21.0	12.2	21.5	16.4
24/10-25/10 . . . . .	24.1	21.8	9.1	21.3	—
25/10-26/10 . . . . .	28.1	22.3	—	23.7	22.0
Average . . . . .	21.4	18.9	9.1	20.7	18.8
C. Averaging only those experiments from Part B in which corresponding alternatives in air and N <sub>2</sub> are available.					
	20.7	15.0		19.4	17.0
	16.3	14.2		19.7	18.4
	19.1	17.3		21.7	21.6
	24.1	21.8		19.9	17.2
	28.1	22.3		21.5	16.4
				23.7	22.0
Average . . . . .	21.7	18.1		21.0	18.8

At the end of the season, experiments on the effect of an atmosphere of nitrogen on starch hydrolysis in leaf discs at 5° and 25° C have been made, using dessiccators in order to avoid the eventual harmful influence of the small gas space. These series have not been worked out immediately, and some of the series were lost because the ethanol in which they were stored, had dried up. A drawback, moreover, was, that the guarantee against leakage was considerably smaller in the case of the dessiccators. Table 7 summarizes the available data. A higher illumination standard was used for the starch determinations so that the absolute transmission values are not directly comparable to those of the earlier series. Each figure in the Table is the average of 6 leaf discs. Considerable starch hydrolysis has taken place in all cases. The difference between air and nitrogen is considerably less than in the experiments in which tubes were used. Notwithstanding the fact that the dessiccators were ventilated with pure nitrogen (from bomb, not passing a reduction oven) and in some cases evacuated before, the results are nearer to those obtained in tubes when the gas contains some percents of oxygen. Small leakages of the dessiccators cannot be fully excluded as contributors to this result. The experiments of the effect of oxygen will have to be continued.

#### 4. SUMMARY

Starch hydrolysis in leaves of *Helianthus tuberosus*, a short day variety, and in leaves of *H. annuus*, flowering in long day, was investigated by a simple colorimetric starch estimation *in situ* (§ 2).

Leaf halves, leaf discs, and leaves attached to considerable stem pieces, did not show conspicuous differences in starch hydrolysis. Moreover, the daily course of starch synthesis and breakdown was studied under natural conditions (figs 2, 3).

*The temperature curve of starch hydrolysis mostly shows a pronounced maximum at about 0–3° C, a minimum around 10° C, and a renewed increase at temperatures above 15° C (figs 4–9).*

An attempt has been made to explain the observed behaviour by assuming three processes, all possessing a temperature curve with a normal, positive rate increment with respect to temperature, *viz.*, starch hydrolysis, (re)synthesis of starch, and respiration of sugars (fig. 12).

Experiments on the effect of certain temperature sequences (3°; 13°; 31°, 13°) made in connection with the above tentative explanation of the temperature curve, failed to demonstrate a difference in behaviour at 13° C, depending on the preceding temperature. No re-synthesis of starch at 13° C after hydrolysis at 3° C was found. The reason may be that during a preceding period of hydrolysis at a temperature which is not suitable for (re)synthesis, the sugar concentration is lowered too much by diffusion, thus preventing effective resynthesis (figs 10, 11).

The temperature curve, in *H. tuberosus*, was found to be much the same in leaves of various physiological ages (fig. 9).

In air with low CO<sub>2</sub> content, light of various intensities was found not to influence starch hydrolysis in *H. tuberosus*, at various temperatures (figs 13, 14). In other experiments, 5 % CO<sub>2</sub> in air decreased hydrolysis considerably in light, but also in darkness (at 25° C) (Table 4), and this time, also in air a difference between light and darkness was found.

Lack of oxygen was found to decrease starch hydrolysis, especially at high temperature. (Table 5, 6). In large gas spaces this effect was less than in small ones, but in the large gas spaces used (dissicators) anaerobiosis may not have been fully satisfactory (Table 7). The study of the influence of the composition of the gas phase still is incomplete.

Attempts were made to study the effect of sugar solutions on starch hydrolysis. In many cases a decrease in hydrolysis was found, but it was observed that also water could bring about similar effects, bringing the phenomenon into contact with the effects of lack of oxygen. These experiments, therefore, still were felt to be unsatisfactory.

It was observed occasionally that wilting promotes starch hydrolysis.

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