Observations on the Oxygen Uptake of Isolated Plant Tissue

I. The Effect of Phosphate and of Added Carbohydrate

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With one Figure in the Text.

INTRODUCTION

IN the course of an examination of the possibility of oxygen uptake by the agent of aucuba virus disease of tomato, a number of studies of the behaviour of plant tissue in Barcroft respirometers were made. The experiments with the virus material yielded negative results; in no instance, with crude virus juice filtered through Pasteur-Chamberland L3 candles, or with purified and concentrated virus material (Caldwell, 1935), was there any indication that oxygen was taken up, even when large quantities of active virus-agent were present in the experimental material.

Subsequent experiments, performed with tissue of healthy and diseased plants, while they threw little light on the problem of virus disease, yet gave results which are suggestive of other lines of investigation, and some of these results are recorded in this paper.

MATERIALS AND METHODS

The material used was slices of tomato stem tissue prepared by removing, under sterile conditions, the extra-cambial tissues and then cutting the remaining tissue longitudinally into slices about 2·5 cm. long by 0·5 mm. thick. In preliminary experiments it was found that, if thicker blocks of tissue were used, the oxygen uptake was limited by the rate of diffusion into the block; the type of results obtained are illustrated in Table I.

As filling the Barcroft bottles with oxygen instead of air (to increase the external oxygen concentration) caused no measurable increase in the oxygen uptake of slices 0·5 mm. thick, it was concluded that the slices were sufficiently thin to allow of adequate diffusion of oxygen into the interior of the tissue (cf. Warburg, 1930).

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The apparatus used for the measurement of oxygen uptake consisted of Barcroft differential manometers; the customary technique was employed (Dixon, 1934). All the measurements were made at 26° C. The oxygen uptake is expressed throughout as μl. of gas (dry at N.T.P.) taken up per hour, per gramme fresh weight of tissue.

TABLE I

Comparison of Blocks and Slices

Method of cutting.	Tissue suspended in.	Oxygen uptake (μl./gm./hr.) (1)	(2)	(3)
Whole block. .	M/10 KH_2PO_4	64	56	61
Half blocks . .	,,	73	74	78
Slices . . .	,,	81	78	84
Whole block. .	M/30 KH_2PO_4	—	63	—
	M/30 KH_2PO_4+0·5 per cent. glucose	—	70	—
Half blocks . .	M/30 KH_2PO_4	—	62	—
	M/30 KH_2PO_4+0·5 per cent. glucose	—	79	—
Slices . . .	M/30 KH_2PO_4	—	70	—
	M/30 KH_2PO_4+0·5 per cent. glucose	—	88	—

The procedure in a typical experiment was as follows: the slices of tomato stem, prepared as described above, were divided into six approximately equal batches. Each batch (weighing between 0·1 gm. and 0·7 gm.) was placed in a tared Barcroft bottle containing 3 ml. of sterile dilute potassium dihydrogen phosphate (KH_2PO_4) solution, and the whole weighed. The usual roll of filter-paper and KOH solution were added, the apparatus placed in the bath and brought to equilibrium, and readings were then taken at intervals over a period of six hours. Dilute acid potassium phosphate was used in which to suspend the slices as it is a non-poisonous medium of an acidity comparable with that of tomato juice. In the earlier experiments the concentration of phosphate was varied; in the later experiments, only one concentration of phosphate (M/30) was used, but sugar was added to some of the bottles, the required amount being dissolved beforehand in the phosphate medium.

In all the work recorded in this paper precautions were taken to ensure that no bacterial contamination took place; when on one occasion this did occur the increase in oxygen uptake late in the six-hour period was so marked that it was easy to see that something unusual had happened.

Note on the accuracy of the results.

Every figure for oxygen uptake given in the tables is a mean value, derived from six samples in Tables II and III, and two or three samples in the later tables. The actual results from which the means are taken varied over a fairly wide range. The main factor contributing to this variation was probably the difficulty of obtaining uniform sets of samples of the relatively large size necessitated by the small oxygen

uptake of plant tissue. Where the mean oxygen uptake was above 40 μl./gm./hr., the average standard deviation was found to be about 10 per cent. of the mean. It follows that differences, due to treatment, of less than 10 per cent. of the mean value of the control cannot be detected. Most of the differences reported are of a higher order; significance tests were applied to them all, and those differences which were found not to be significant are so marked in the tables.

Results

1. *Phosphate concentration and age of tissue.*

In the first series of experiments tissue from healthy plants was used; the plants were of two different sizes; plants in the 5th leaf stage (referred to as 'very young' plants), and plants in the 6th to 12th leaf stage (referred to as 'young' plants). The oxygen uptake of tissue from plants of both types was measured in distilled water, and in different concentrations of phosphate. The results are given in Table II.

Table II

Oxygen Uptake of Slices of Tomato Stem (Healthy Plants)

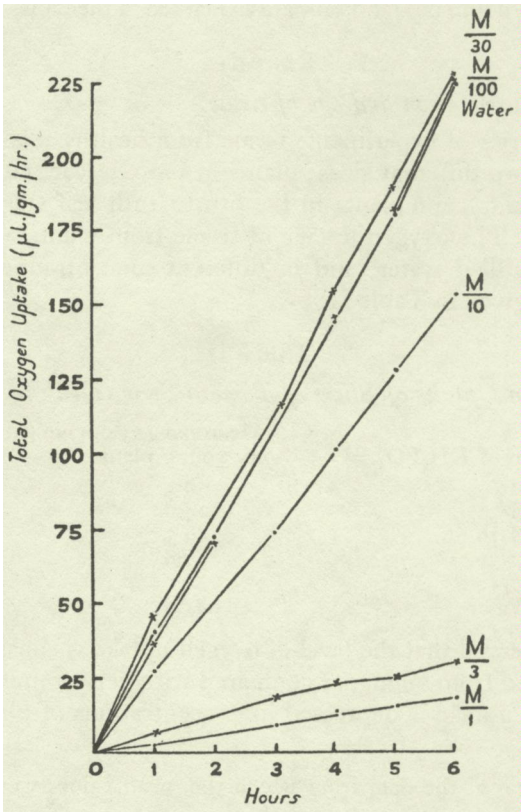
Concentration of KH_2PO_4.	Mean oxygen uptake (μl./gm./hr.). Very young plants.	Young plants.
M	8·1	—
M/3	14·0	39·5
M/10	59·5	114·2
M/30	87·8	137·4
M/100	86·0	112·3
Water	85·9	114·5

It will be observed that the level of oxygen uptake is consistently higher in the tissue derived from young, as compared with very young, plants, and also that the oxygen uptake is depressed in concentrations of phosphate stronger than M/30.

[An examination of the data from which the mean values were derived showed that (*a*) the mean value for oxygen uptake at any concentration of phosphate from M to M/30 was significantly different from the means at adjacent concentrations, and (*b*) that the value given by tissue from very young plants at any concentration of phosphate was significantly different from the value given by tissue from young plants at the same concentration.]

In the diagram are given progress curves for the oxygen uptake of individual samples of about equal weight, one from each experiment of the first series; it will be seen that the depressing action of the stronger solutions of phosphate is apparent throughout the period of observation. It will also be observed that the rate of oxygen uptake is very nearly constant in every case (i.e. the 'progress curves' approach very closely to straight lines), and is maintained at the same level right to the end of the six-hour period. This constant rate of oxygen uptake appears to be characteristic of the stem tissue, as it was observed in every sample throughout the experiments. Marked curling of

the stem slices was observed to take place in the stronger solutions of phosphate (M to M/10); but the slices never became flaccid in the six-hour period, and indeed retained their normal turgidity even after standing for twenty-four hours.



Very young plants. Oxygen uptake of individual samples of tissue in water and in various concentrations of acid potassium phosphate.

The first series of experiments was then repeated exactly with plants infected with aucuba mosaic virus, and in addition a few experiments were performed with tissue from old diseased plants (i.e. plants that had flowered.)

It will be seen that the results agree with those obtained with healthy plants, both in the depression of oxygen uptake in the stronger concentrations of phosphate, and in the consistently lower level of respiration in tissue from plants in the 5th leaf stage, as compared with slightly older plants.

As might be expected, the tissue from old plants has a still lower level of respiration (cf. Kidd, West, and Briggs, 1921). But the low level of oxygen uptake in tissue from very young plants, observed with both healthy and diseased plants, is a more unexpected result.

That a low respiration rate is not an intrinsic property of young tissue is shown by a consideration of the results obtained when samples taken from the apex of the stem of a plant in the 6th to 12th leaf stage are compared with samples taken farther and farther down the stem. A plant in this stage is

TABLE III

Oxygen Uptake of Slices of Tomato Stem (Diseased Plants)

Concentration of KH_2PO_4.	Mean oxygen uptake (μl./gm./hr.).		
	Very young plants.	Young plants.	Old plants.
M	13·9	—	14·3
M/3	27·2	55·7	35·0
M/10	57·7	102·7	54·3
M/30	78·0	121·1	47·8
M/100	*100·5	109·0	—
Water	*88·1	106·7	—

* (2 samples only.)

TABLE IV

Position Gradient of Respiration

Position on stem.	No. of samples.	Mean oxygen uptake (μl./gm./hr.).
Apex . . .	5	163
2nd internode .	6	148
Middle part . .	3	97
,, . .	3	124
,, . .	3	114
,, . .	3	104
Near ground-level.	3	98

still actively growing, and the apex of the stem is composed of more recently formed, and therefore younger, tissue than the lower part of the stem.

The results of one such experiment are given in Table IV; a single well-grown healthy young plant was used, and the oxygen uptake of samples taken farther and farther down the stem measured in M/30 KH_2PO_4.

In a similar experiment, with a virus plant of the same age, samples of tissue from the apex of the stem had a mean oxygen uptake of 169 μl./gm./hr., while the main part of the stem gave mean values of 100–120 μl. So in both cases the youngest tissue, taken from the apex of the stem, had a much higher respiration rate than the slightly older tissue from the lower part of the plant.

It is therefore unlikely that the low level of oxygen uptake in tissue from plants in the 5th leaf stage was caused by the youth *per se* of the tissue, and another cause must be looked for. Among the probable causes is a lack of available respiratory substrate; to obtain further information on this point the experiments reported below in section 2 of this paper were carried out.

2. *The effect of added carbohydrates.*

A series of experiments was carried out to test the effect of adding carbohydrate to tissue from plants of different ages. The carbohydrates used were the monosaccharides glucose and fructose; their effect on the oxygen

TABLE V

Addition of Glucose to Tissue from Old Plants

Type of plant.	Glucose per cent.	Mean oxygen uptake (μl./gm./hr.). Control.	Treated.	Increase per cent.
After flowering .	0·5	95	99	5 (not sig.)
,, .	0·5	95	95	0
Very old . .	0·5	48	56	15 (not sig.)

TABLE VI

Addition of Carbohydrate—Young Plants (Healthy)

Carbohydrate.	Concentration per cent.	Mean oxygen uptake (μl./gm./hr.). Control.	Treated.
{Glucose . .	0·2	*96	96
{Fructose	0·2	—	98
Glucose . .	0·2	133	135
,, . .	1·0	143	153
Fructose . .	1·0	163	161
Glucose . .	0·5	103	105 (plant 132 hours in dark).

* (2 samples).

uptake of tomato tissue appeared to be identical (except in one anomalous experiment which we were unable to confirm).

[M/30 acid phosphate was used as the suspension medium, as it had already been found to give the maximum oxygen uptake; in each experiment a sugar solution of the required strength (0·2, 0·5, or 1·0 per cent.) was made up in sterile phosphate and added to three of the Barcroft bottles, while phosphate alone was added to the other three, thus providing a separate control for each experiment. (If glucose and fructose were used in the same experiment, they were added to two bottles each, leaving two control samples.)]

In the first set of experiments old plants were used; only a limited amount of material, and that all from virus plants, was available. The results, given in Table V, indicate that a negligible increase of oxygen uptake was obtained on the addition of glucose, although the uptake of the control samples was small.

When 'young' plants (in the 6th–12th leaf stage) were used, the level of oxygen uptake in the controls was rather higher; but in this case also there was not a significant increase in oxygen uptake on the addition of carbohydrate. Some typical results are given in Table VI, which includes two experiments with a 1 per cent. concentration of sugar, and one where the plant was kept in the dark before the experiment.

When tissue from very young plants (5th leaf stage) was used, on the other

hand, a very marked increase in oxygen uptake was produced in every case by the addition of carbohydrate. The results of some experiments with healthy 5th leaf-stage plants are given in Table VII. In the later experiments of this set the plant was kept in the dark for some time before the experiment;

TABLE VII

Very Young Healthy Plants—Addition of Carbohydrate

Hours in dark.	Carbohydrate.	Concentration per cent.	Oxygen uptake (μl./gm./hr.). Control.	Treated.	Increase per cent.
0	Glucose	0·2	*84	107	27
	Fructose	0·2	—	107	27
0	Glucose	0·2	60	105	75
0	Glucose	0·2	68	124	82
16	Glucose	0·2	*46	65	46
	Fructose	0·2	—	67	50
40	Glucose	0·5	*47	79	58
	Fructose	0·5	—	76	59
48	Glucose	0·5	46	88	91
64	Glucose	0·5	*37	84	127
	Fructose	0·5	—	67	80
72	Glucose	0·5	41	59	44

* (2 samples).

TABLE VIII

Very Young Diseased Plants—Addition of Glucose

Hours in dark.	Concentration per cent.	Oxygen uptake (μl./gm./hr.). Control.	Treated.	Increase per cent.
16	0·5	19	51	170
96	0·5	34	83	144
200	0·5	24	46	100

this treatment reduced the oxygen uptake of the controls, and augmented slightly the effect of added carbohydrate. The increase in oxygen uptake is expressed as a percentage of the control uptake; i.e. by the expression $\frac{(b-a)\ 100}{a}$, where a is the mean oxygen uptake of the control, and b of the treated, tissue.

Owing to a shortage of material only a few experiments could be performed on diseased 5th leaf-stage plants; the results are given in Table VIII.

These three experiments, as far as they go, show that the oxygen uptake of the control tissue is reduced to a greater extent in diseased than in healthy plants, by keeping the plant in the dark before the experiment, and that the addition of glucose produces a proportionately greater rise in oxygen uptake.

Respiratory quotient.

Some measurements of the respiratory quotient were performed on tissue from healthy young plants. The technique used was that described by Dixon

(1934), in which the measurements of oxygen uptake, carbon-dioxide output, and bound carbon dioxide are performed on three different samples; the estimate of the respiratory quotient is therefore only approximate.

TABLE IX

Respiratory Quotient

Expt. no.	Oxygen uptake (μl./gm./hr.).	R.Q. (CO_2/O_2).
1	137	0·94
2	184	1·02
3	158	1·03
4	158	1·02
5	144	1·01

None of the figures obtained for the respiratory quotient was significantly different from unity.

DISCUSSION

The method employed in this work appears to give, with a very small amount of material, an adequate measurement of the respiration of plant tissue. That the measurements of oxygen uptake recorded were in fact due to normal aerobic respiration is indicated by the following observations: (*a*) the slices of stem tissue were invariably found to take up oxygen at a constant rate which was maintained over the whole period of an experiment; (*b*) the estimates obtained of the respiratory quotient approached closely to unity; (*c*) the tissue slices used showed no sign of flaccidity after standing for twenty-four hours.

The effect of phosphate.

As the oxygen uptake of slices suspended in distilled water was only slightly less than that of slices suspended in M/30 phosphate, it is evident that there was no question of phosphate starvation in the tissue used. M/30 phosphate was probably effective in producing the largest oxygen uptake observed because it approached most nearly, in acidity and tonicity, to the cell-sap of all the solutions used. It certainly caused less curling of the tissue slices than the other solutions. The marked depression of respiration observed in the presence of M/10 or stronger concentrations of phosphate is probably due to their being hypertonic to the cell-sap; it is interesting to compare with our results those of van Heyningen (1935) on the oxygen uptake of a variety of animal tissues. Van Heyningen criticizes the work of Dixon and Elliott (1929) on the ground that they used a buffer solution containing M/10 phosphate in their experiments, and that this concentration was too strong; the medium that he used contained M/40 phosphate, and in this medium he obtained consistently higher values of oxygen uptake than those recorded by Dixon and Elliott for the same animal material.

The effect of age on oxygen uptake.

In any study on the effect of age on the respiration of isolated parts of a plant it is important to distinguish between the age of the plant from which the part has been removed and the stage of development reached by that particular part. That a failure to make this distinction may lead to misconceptions of the effect of age on physiological processes has been shown by Richards, who points out (1934) the fallacies underlying the method of estimating the effect of age on, e.g. respiration, by performing measurements on successive leaves taken from the same plant, and states that corresponding leaves from plants of different ages must be used in such an estimation, for, unless this is done 'it is impossible to separate effects which may be possibly ascribed to age as such from those due to change in conditions of nutrition, etc.' This point is emphasized in the work reported in this paper; for instance, the extreme top of a plant in the 6th to 12th leaf stage may be regarded as young tissue in one sense, as it is the last formed part of the plant; but we have shown that it has a very much higher rate of respiration than tissue removed from a very young plant. The tissues at the top of a mature plant are not physiologically equivalent to those of a very young plant.

In a well-grown young plant there must be an ample supply, throughout the plant, of respirable material formed by photosynthesis, and under these circumstances very young tissue exhibits a higher rate of oxygen uptake than older tissue. It is therefore probable that the low rate of oxygen uptake exhibited by tissue from very young plants is caused by a lack of available substrate, most of the respirable material having been used up in tissue formation, and not by a lack of activity in the respiratory enzyme system.

This suggestion is confirmed by the results obtained on the addition of sugar to the respiratory tissue. In young plants, where both an efficient respiratory system and an ample supply of respiratory substrate are present, the oxygen uptake of untreated tissue is high and is not raised by the addition of carbohydrate. In both very young and very old plants the oxygen uptake of untreated tissue is low, but from different causes. In old plants the uptake is only very slightly raised by added carbohydrate, and the low respiration rate is presumably due to a decline in the activity of the respiratory system. In very young plants the respiratory system is highly active, but there is a lack of available substrate, and accordingly the addition of glucose or fructose produces a very marked rise in oxygen uptake.

The results obtained with added carbohydrate also indicate the one point of difference between healthy- and virus-diseased tissue that was observed in these experiments. It appears that respiratory substrate is lost to a greater extent from virus plants when they are kept in the dark than from healthy plants. Glucose, added to the tissue from starved virus plants, produced a greater proportional increase of respiration than in the corresponding normal tissue. The incompleteness of these results prevents any very definite conclusion being drawn from them, but they partly confirm a suggestion made

by Caldwell (1934) that the enzymes concerned in the preliminary stages of respiration were more active in virus-diseased than in healthy plants. The preliminary experiments, with extracted and with purified juice from virus plants, gave no indication that the virus agent itself had any oxygen uptake. This confirmed the view that the increase in the respiration rate of the tissues of diseased plants was not associated with the respiration of the virus agent itself.

Summary

1. The oxygen uptake of thin slices of tomato stem tissue was measured in Barcroft respirometers, and found to be maintained at a constant rate over a six-hour period.

2. The highest values for oxygen uptake were observed in presence of M/30 potassium dihydrogen phosphate; measurements in distilled water gave slightly lower values, and stronger solutions of phosphate produced a marked depression of oxygen uptake.

3. Tissue from very young plants, in the 5th leaf stage, showed a lower level of oxygen uptake than tissue from slightly older plants, up to the 12th leaf stage. A low level of oxygen uptake was also observed in tissue from old plants that had flowered.

4. The small oxygen uptake of tissue from very young plants was markedly raised by the addition of glucose or fructose, but no such rise was observed on adding sugar to tissue from very old plants.

5. It is concluded that the oxygen uptake is limited in old plants by the activity of the respiratory enzyme system, and in very young plants by the amount of available respiratory substrate.

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