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## Researches on Plant Respiration

### IV—The Relation between the Respiration in Air and in Nitrogen of certain Seeds during Germination. (b) Seeds in which Carbohydrates Constitute the Chief Food Reserve

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#### INTRODUCTION

Part III of this series (Leach and Dent, 1934) dealt with experimental data concerning the effect on the respiration rates and respiratory quotients of certain fat-storing germinating seeds when successively surrounded by atmospheres of air, nitrogen, and air. The present paper describes similar data relating to the respiration of germinating seeds in which the predominant food reserves are carbohydrates.

The seeds used in the present investigation, as with those used in the previous one, were of species belonging to genera widely separated from one another phyletically. The seeds were all obtained from Messrs. Sutton & Sons of Reading and the species and varieties used were as follows:—

*Fagopyrum esculentum*, *Zea mais* (Sutton's Improved Japanese Striped), and *Lathyrus odoratus* ("What Joy").

The seeds of all three species are similar in the respect that in each case starch forms the chief reserve food material.

The experimental data were all obtained by the katharometer method already described (Stiles and Leach, 1931; Leach, 1932) the experimental seedlings being maintained at a constant temperature of 25° C, and under other conditions identical with those used for *Ricinus*, *Helianthus*, and *Cucurbita*. Respiration rates are expressed as milligrams carbon-dioxide

output per gram fresh (unsoaked) weight of seed (without seed coat) per hour.

## EXPERIMENTAL RESULTS

### 1—*Lathyrus odoratus*

The normal course of respiration, and the changes undergone by the respiratory quotient, during the early stages of germination of seeds of *Lathyrus odoratus* have been described and discussed at length in earlier papers of this series (Stiles and Leach, 1931, 1933). They are shown in fig. 1 which indicates the result of a recent experiment using three seeds

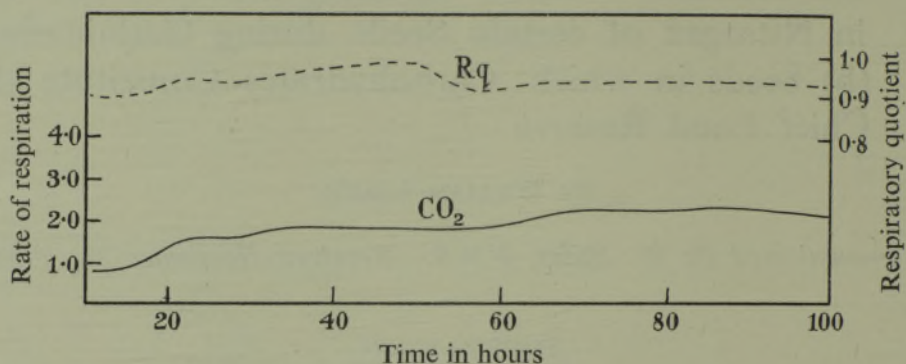


FIG. 1—*Lathyrus*, experiment 204.

with their testas previously removed. It will be noted that during the first 100 hours of germination, the output of carbon dioxide rises at a somewhat irregular rate to a maximum value, and having reached this maximum, it then begins to decrease. The respiratory quotient during this period appears to fluctuate somewhat but is, however, usually in the neighbourhood of 0.90.

The respiratory behaviour of the germinating seeds (without testas) of this species when the gas surrounding them is changed from air to nitrogen, and later from nitrogen back to air, is shown in Table I and fig. 2.

It will be observed from these that the change from air to nitrogen causes a rapid decrease in carbon dioxide output to an initial minimum rate. The value of this minimum rate as experimentally recorded varies between one-third and one-half of the rate recorded immediately before the air was replaced by nitrogen.

Following on this initial fall, the production of carbon dioxide increases for some hours until a maximum is reached, after which it again decreases, continuing to do so throughout the remainder of the period during which



TABLE I—THE RESPIRATORY BEHAVIOUR OF *Lathyrus odoratus* IN AIR AND IN NITROGEN

Experiment number											
168			170			171			203		
One seed used			One seed used			One seed used			Three seeds used		
Age of seedling in hours	Respiration rate mg CO <sub>2</sub> /gm/hour	Rq	Age of seedling in hours	Respiration rate mg CO <sub>2</sub> /gm/hour	Rq	Age of seedling in hours	Respiration rate mg CO <sub>2</sub> /gm/hour	Rq	Age of seedling in hours	Respiration rate mg CO <sub>2</sub> /gm/hour	Rq
58.5	Air	0.81	69.0	Air	0.87	100.5	Air	0.91	56.0	Air	0.88
64.5	1.08	0.81	87.0	1.16	0.88	105.5	2.71	0.91	62.0	1.21	0.89
71.0	1.18	0.84	90.5	Nitrogen		110.5	2.41	0.90	68.0	1.14	0.89
76.0	1.18		92.0	0.72		114.5	2.16		71.5	1.06	0.89
78.5	0.49		93.0	0.39		116.0	Nitrogen		72.5	0.76	
82.5	0.64		94.0	0.27		117.0	0.78		74.0	0.58	
86.0	0.67		96.5	0.45		121.5	1.13		76.0	0.55	
89.0	0.51		102.5	0.50		124.5	1.18		78.0	0.49	
93.5	0.51		109.5	0.66		128.5	1.41		80.5	0.61	
95.5	Air	0.82	115.0	0.59		134.0	1.48		83.5	0.65	
97.5	1.09	0.72	123.5	0.52		144.0	1.41		86.5	0.74	
100.5	1.04	0.68	135.5	0.54		156.5	1.11		89.5	0.65	
104.5	1.08	0.79	146.0	0.47		167.0	0.89		93.0	0.67	
112.0	1.47	0.86	174.0	0.41			Air	0.88	94.0	Air	0.95
119.5	1.80	0.85	182.5	0.37		169.0	2.44	0.83	95.5	0.95	0.89
130.5	1.42	0.85	187.5	0.37		172.0	2.61	0.80	97.5	0.98	0.60
	1.41		190.5	Air		175.5	2.52		99.5	1.08	0.72
			192.5	1.13	0.70	181.0	2.24	0.94	101.5	0.98	0.79
			194.5	1.24	0.70	189.5	2.89	0.97	103.5	1.35	0.89
			196.5	1.01	0.64	195.5	3.78	0.97	106.5	1.39	0.89
			198.5	1.19	0.67	201.0	3.67		110.5	1.39	
			200.5	1.35	0.69				116.0	1.29	
			205.5	1.50	0.70				121.0	1.10	0.89
			209.0	1.65	0.75				125.0	1.04	0.88
			214.0	1.43	0.87				129.0	1.06	0.89
			217.0	1.55	0.95				133.0	1.12	0.90
			229.5	2.32	1.00				137.0	1.20	0.91

the seedlings are kept in nitrogen. The falling off in the rate of carbon dioxide production, from this maximum value in nitrogen, is slow and in only one of the experiments performed (namely experiment 170 in which the nitrogen period was prolonged to 90.5 hours) was its final recorded value lower than the initial minimum referred to above.

The change to respiration in air, after the period in nitrogen, results in a rapid rise in carbon dioxide output until this is approximately the same as it originally was before the change from air to nitrogen was made. A further and usually more gradual rise in carbon dioxide output then follows until a maximum value is reached after which the rate decreases for a time and then again increases. This final increase seems to be the settling down of the respiration curve to its normal course for seedlings

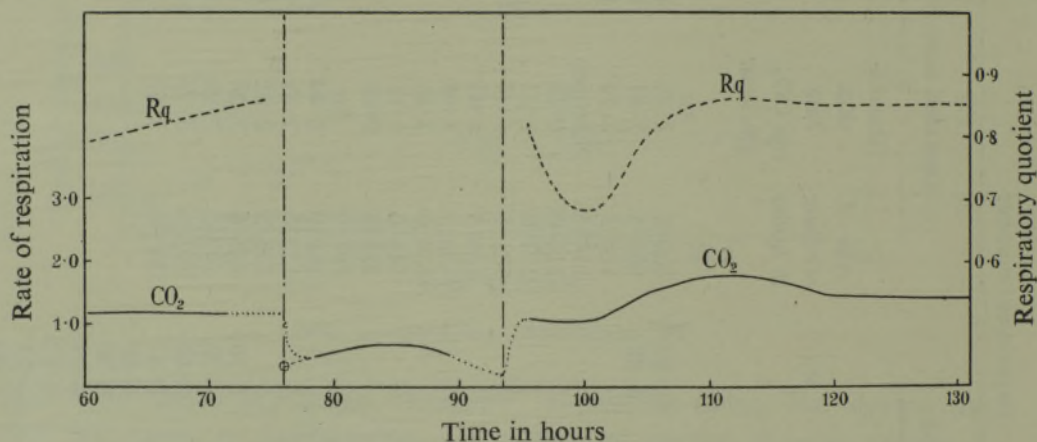


FIG. 2—*Lathyrus*, experiment 168.

respiring in air, the form of which is determined by the developmental stage in which the particular seedling happens to be.

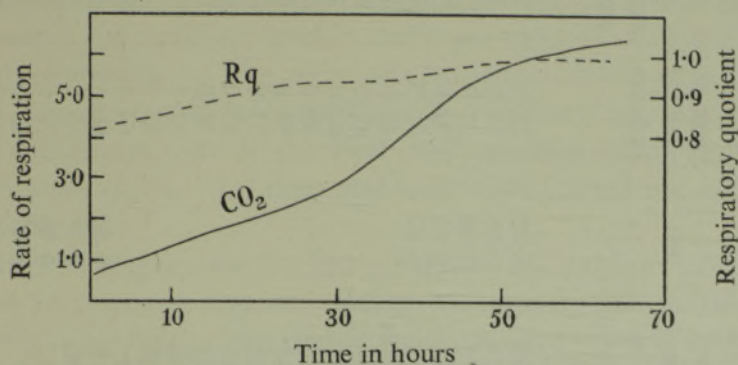
The effect of the period in nitrogen on the respiratory quotients of the seedlings, when they are again respiring in air is similar to that found for fat-storing seeds. The change from anaerobic to aerobic conditions is followed by a relatively high rate of oxygen intake as compared with carbon dioxide output. The beginning of the second period in air is characterized by a falling respiratory quotient. This fall is continued for a period of time varying between 5 and 10 hours until a minimum value is reached. As will be seen from the data given in Table I this minimum value lies more commonly between 0.60 and 0.70 although in experiment 171 the lowest value recorded was only 0.80.

After reaching this minimum the respiratory quotient rises to a level approximating to unity and thus takes up its normal value for aerobic respiration.



2—*Fagopyrum esculentum*

The course of respiration and the changes taking place in the value of the respiratory quotient, during the early period of germination in air, of seeds of *Fagopyrum* are shown in fig. 3. From the start the carbon dioxide output increases at a comparatively rapid rate, while the respira-

FIG. 3—*Fagopyrum*, experiment 119.

tory quotient rises from an initially low value practically to unity (Stiles and Leach, 1933).

The interruption of this normal behaviour, by the introduction of a period of anaerobiosis in nitrogen, results in disturbances which show certain resemblances to, and certain differences from, those already described for *Lathyrus*.

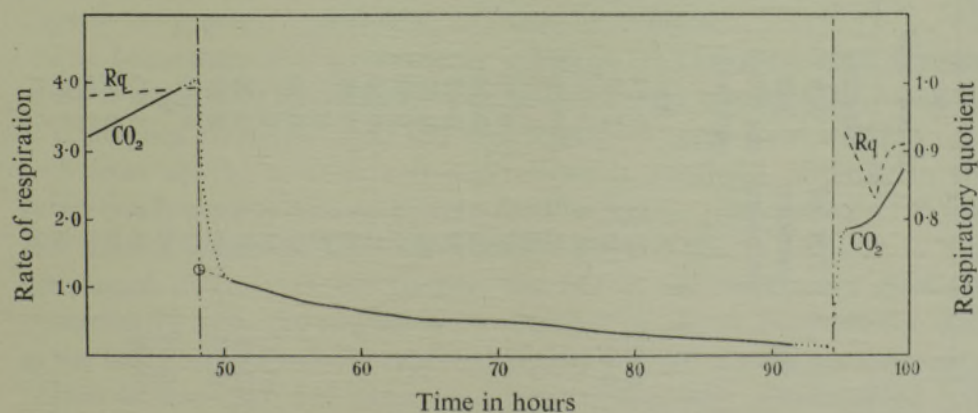
FIG. 4—*Fagopyrum*, experiment 191.

Table II sets out the numerical details of four experiments performed on germinating seeds of *Fagopyrum*, and the results of one of these experiments are shown graphically in fig. 4.

An examination of these data shows that on changing from air to nitrogen, the usual rapid fall in carbon dioxide output takes place, this





being followed by a slower rate of decrease. In two experiments, namely numbers 201 and 202, this decrease is followed by a very definite rise and a second fall similar to that observed with *Lathyrus odoratus*. In experiment 186 this rise which occurs between the 34th and 38th hours is only very slight, while in experiment 191 it was not recorded at all. No explanation can be offered to account for this difference between the behaviours of the seedlings used in these two pairs of experiments beyond that it may possibly be due to an after-ripening effect; experiments 186 and 191 were respectively carried out in February and March of 1934 and the seeds used were of the 1933 crop, whereas experiments 201 and 202 used seeds of the 1934 crop and were both carried out in November of that same year.

Seedlings of *Fagopyrum* on being restored to aerobic conditions after a period in nitrogen appear quickly to resume their normal aerobic respiratory behaviour. The change from nitrogen to air is marked by the usual rapid rise in carbon dioxide output which has been observed in the other species used in these investigations. This rise continues until the rate approaches that recorded at the time the change from air to nitrogen was made. A slower rise in carbon dioxide output follows as the normal aerobic course of respiration of the seedlings is gradually resumed. The definite rise to a maximum and subsequent temporary fall in the respiration rate at this stage of the experimental treatment, so pronounced in *Lathyrus*, is only shown very slightly in one of the experiments with *Fagopyrum* (namely experiment 202 after 68·5 hours).

The behaviour of the respiratory quotient on changing from nitrogen to air is essentially the same for *Fagopyrum* as was recorded for *Lathyrus*. It undergoes an initial rapid fall to a minimum value from which it rises and takes up the normal course it follows in seedlings respiring in air, being finally maintained at or very close to unity. Experiments 186 and 191 show a difference from experiments 201 and 202 in addition to that mentioned above. In the former, the fall in the respiratory quotient, immediately after the change from nitrogen to air, is much slower than in the latter, in fact in both experiments 201 and 202 it took place during a period of less than 1·5 hours and was consequently not recorded. In experiments 186 and 191 it extended over approximately 3·5 hours and 3·0 hours respectively.

### 3—*Zea mais*

Experimental data for *Zea mais* of a corresponding nature to those already given and dealt with for *Lathyrus* and *Fagopyrum* are set out in

Table III. The normal course of respiration and the changes in the respiratory quotient during the germination of a single seed of the variety used in these experiments are shown in fig. 5. The respiratory behaviour of a seedling under successive aerobic and anaerobic experimental conditions is shown in fig. 6.

Replacement of the air surrounding the seedlings by nitrogen produces a similar effect on their carbon dioxide output to that produced when *Lathyrus* seedlings are used. The rate of carbon dioxide output falls

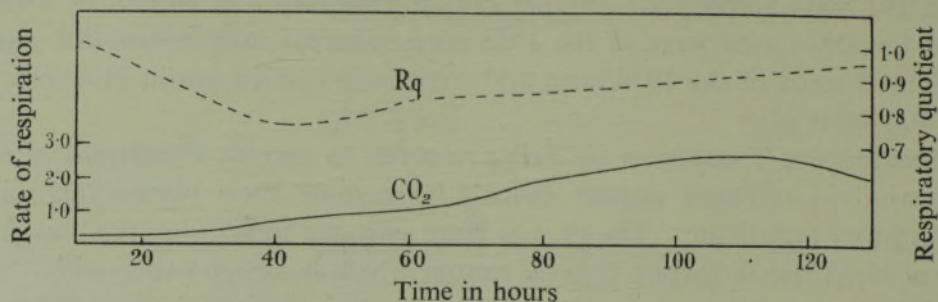


FIG. 5—*Zea mais*, experiment 192.

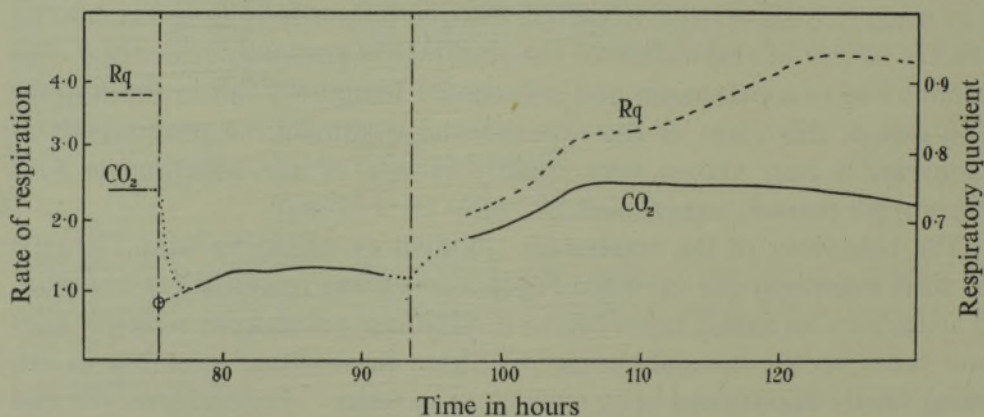


FIG. 6—*Zea mais*, experiment 181.

to a minimum value from which it rises to a maximum and then again falls.

The change from nitrogen to air at the end of the experimental period in nitrogen results in a rapid rise in the carbon dioxide output which then more gradually assumes its normal aerobic course. At this stage of the treatment the behaviour of *Zea mais* resembles that of *Fagopyrum* rather than *Lathyrus* as no definite rise to a maximum and subsequent fall, before the resumption of the normal course, was recorded.

The behaviour of the respiratory quotient during these last aerobic stages presents no marked difference from what occurs in the other two





species dealt with in this paper. At the beginning of the final aerobic period the respiratory quotient falls rapidly to a relatively low value, after which it follows a somewhat irregular rising course until normal behaviour is resumed.

### DISCUSSION

The investigations described in this paper form a direct continuation of those described in Part III (Leach and Dent, 1934). It is not therefore proposed to restrict this discussion to a consideration of the experimental results obtained with carbohydrate-storing seeds only, but to deal briefly with both carbohydrate and fat-storing seeds.

The three starch-storing seeds, namely those of *Lathyrus*, *Zea*, and *Fagopyrum* show a very similar respiratory behaviour. In all three the change from respiration in air to respiration in nitrogen is marked by a rapid and considerable fall in the output of carbon dioxide. The initial low rate of anaerobic carbon dioxide production in all three seeds is followed by a rise and subsequent fall. This last fall, as shown in experiments 170 and 171 with *Lathyrus* and more definitely in experiments 186 and 191 with *Fagopyrum*, appears to continue throughout the period during which the seeds are kept without oxygen.

The change from anaerobic respiration in nitrogen to aerobic respiration, produces an immediate rise in the carbon dioxide output. In *Lathyrus* this rise continues to a maximum from which it then falls for a few hours, this fall being followed by a more gradual rise, as the normal aerobic rate of respiration of the seedling is gradually resumed. In *Zea* and *Fagopyrum* this early maximum does not occur when the change from nitrogen to air is made, instead, the carbon dioxide output merely increases until the respiration of the seedlings again follows its normal course.

The behaviour of the respiratory quotients in these three carbohydrate-storing seeds, when the change from anaerobic to aerobic conditions is made, corresponds to that observed with fat-storing seeds. The respiratory quotient first falls, usually rapidly, and then rises more gradually to its normal value for the particular seedling under observation. There is thus evidence that in carbohydrate-storing seeds also, some easily oxidizable substance accumulates during anaerobiosis, which, when oxygen is again supplied, is rapidly respired. In *Lathyrus* it would appear that this substance is sufficiently active, or accumulates in sufficient quantity, to cause the rate of carbon dioxide output to rise to a value that cannot be maintained, thus causing the peak in the respiration rate referred to above. It is of interest to find that Jensen (1923) records a



similar temporary abnormal aerobic respiration rate for seedlings of *Sinapis alba* after they had been subjected to a period of anaerobiosis in hydrogen.

It will now be of some interest to consider in more detail the respiratory behaviour of the seedlings under investigation, when they are first brought from air into nitrogen. This behaviour in the case of apples was used by Blackman (1928) for the purpose of deducing their rates of glycolysis at the times when the changes from air to nitrogen were made. Therefore, using the graphs constructed from the data obtained from each of the experiments carried out in the present investigation, values were obtained by extrapolation for the rates of carbon dioxide output in air (OR) and in nitrogen (NR) at the moments when the changes from air to nitrogen were made. These values were used in the working out of the NR/OR ratios for each species examined and which are given in Table IV.

If the values of NR/OR for the three carbohydrate seeds are examined it will be noted that the highest figure obtained is 0.37 for *Fagopyrum*, experiment 202, while in the remaining experiments with this species and in the experiments with *Zea* and *Lathyrus* values for NR/OR range down to 0.17.

A point of considerable interest is the close approximation to 0.33 shown by NR/OR in all four experiments with *Fagopyrum*. From this it may be assumed that in *Fagopyrum* the output of carbon dioxide in nitrogen (NR) is one-third of the output in oxygen (OR) at the moment when the transition from oxygen to nitrogen occurs. In other words the change is exactly what would take place if the complete oxidation of hexose to carbon dioxide and water were replaced by its anaerobic breakdown to carbon dioxide and alcohol. In *Fagopyrum* seedlings then (using Blackman's terminology),

$$\text{Glycolysis (Gl)} = \text{Aerobic respiration (OR)}$$

and therefore no oxidative anabolism takes place.

Oxidative anabolism also appears to be absent from the respiratory processes of *Lathyrus* and *Zea*, as with neither of these species does NR/OR ever exceed 0.33. It is not clear why, in the seedlings of these latter, NR/OR shows such low values. The explanation may be that the values of NR adopted for these two seedlings are perhaps too low owing to the difficulty of making correct extrapolations. This is because of the complications in their respiration curves, caused by the pronounced rise in carbon dioxide production in nitrogen which follows the initial transitional fall.

A possible reason why, in *Fagopyrum*, the value of NR/OR so closely approximates to theoretical expectations, may be connected with the fact that the seed of this species is a relatively simple morphological type. It is small, non-endospermic with epigeal cotyledons; and the food store consists almost entirely of starch. When germination occurs practically the whole of the embryo is composed of growing tissues, and owing to its small size the diffusion paths of respiratory gases are short.

TABLE IV

Seedling	Experiment No.	$\frac{\text{NR}}{\text{OR}}$
<i>Lathyrus odoratus</i> .....	168	0.25
	170	0.18
	171	0.25
	203	0.20
<i>Fagopyrum esculentum</i> .....	186	0.36
	191	0.31
	201	0.36
	202	0.37
<i>Zea mais</i> .....	177	0.29
	178	0.28
	181	0.26
	205	0.17
<i>Helianthus annuus</i> .....	172	0.52
	194	0.58
	195	0.57
	196	0.42
<i>Cucurbita pepo</i> .....	179	0.41
	187	0.37
	190	0.42
<i>Ricinus communis</i> .....	184	0.43
	185	0.40
	198	0.72

Turning now to a further consideration of the experiments with fat-storing seeds, it will be seen from Table IV that the NR/OR values for *Ricinus*, *Cucurbita*, and *Helianthus* are all greater than 0.33. It therefore may be possible that, in these, a certain amount of oxidative anabolism takes place.

One tentative suggestion that may be put forward as a partial explanation of the high values of NR/OR in fat-storing seeds is that oxygen supply



may be a limiting factor which prevents the output of carbon dioxide in air reaching its full rate. We know from published chemical data (Pierce, Sheldon, and Murlin, 1933) that reserve fat in seeds is converted to sugar during germination, and that oxygen is absorbed and used in this conversion. In the germinating seeds of *Ricinus*, *Cucurbita*, and *Helianthus*, the low respiratory quotient values indicate that oxygen is being used in this way for the conversion of fat into sugar, as well as for the respiratory oxidation of the latter substance. Blackman (1930) has stated that in respiring apples carbon dioxide production "varies with every alteration of oxygen concentration in the environment". It may therefore be that, with these two processes going on in the same tissue and at the same time, the diffusion rate of oxygen, to the seats of the reactions involved, may be insufficient to allow the reactions to proceed at their maximum rates. This being so, the experimentally obtained values for the rates of carbon dioxide production in air would be something lower than three times those for the rates in nitrogen and thus NR/OR would have values greater than 0.33. A further reference to this point, however, will be made in a later paper of this series when some new experimental data which appear to throw light upon it are discussed.

The values of NR/OR obtained by extrapolation, at the moments when the gas surrounding the seedlings was changed from nitrogen to air, have been examined. Owing to the continued decrease in carbon dioxide production which always finally sets in when the seedlings are kept in nitrogen, and to the already mentioned accumulation of some substance which will readily combine with oxygen, these final NR/OR values show great variation in magnitude in different experiments. They therefore cannot be used for the purpose of confirming the above deductions obtained with the values given by the ratio at the transition from air to nitrogen.

Attention is now drawn to the rise which occurs in the anaerobic production of carbon dioxide, in carbohydrate seeds, immediately following the initial fall which takes place when the change from air to nitrogen is made. This rise is very definitely shown in the experiments with *Lathyrus* and *Zea* and is slightly indicated in those with *Fagopyrum*. Although chemical data are necessary for a final explanation of this rise, it suggests that in the absence of oxygen, some new process which involves the production of carbon dioxide, comes into operation and gradually assumes its maximum rate. It would appear that this process operates on a substrate which is only formed when oxygen is present and is thus, in absence of oxygen, limited in quantity. The activity of the process thus increases to a maximum, only to decrease again as the available supply



of substrate is used up. This rise and fall in the rate of the process is indicated by the rise and fall in the carbon dioxide production which results from it.

The sequence of events which follows, when seedlings of *Lathyrus*, *Zea*, and *Fagopyrum* are transferred from air to nitrogen, may therefore be visualized very broadly as three phases which are outlined below.

- I. The suspension of the oxygen supply brings about a change over from a complete oxidation of hexose to carbon dioxide and water, to its partial oxidation to carbon dioxide and alcohol. This change causes the carbon dioxide production in nitrogen to fall to one-third of what it was in air.
- II. At the moment when the oxygen supply is cut off, the process, which has been discussed above, comes into operation. The activity of this process increases to a maximum and then decreases as its specific substrate diminishes in quantity. With this is associated an output of carbon dioxide which is independent of that referred to in connexion with phase I. This additional carbon dioxide production first rises and then falls as the reactions to which it owes origin, increase and then decrease in velocity.
- III. As the respective substrates for both phases I and II are dependent on oxygen for their formation, they decrease in quantity in absence of oxygen. This decrease in substrate is indicated by the final decrease in anaerobic carbon dioxide output with time, which occurs when seedlings are kept in nitrogen for prolonged periods.

If the experimental data obtained with *Helianthus*, *Cucurbita*, and *Ricinus* are examined, evidence supporting the existence of phase II is not to be found. It may be that, in fat-storing seeds, oxygen supply during respiration in air, acts as a limiting factor on the production of substrate for this second anaerobic phase, in a similar way to that suggested as accounting for the high NR/OR values. The result being that the necessary substrate is absent or is only formed in quantities too small to give experimental evidence of its presence.

Chemical changes taking place in germinating seeds are being investigated in this department and until data regarding these are available the advisability of carrying the present discussion further is doubtful. It appears certain, however, that the respiratory processes of the actively growing tissues of germinating seeds differ in important details from those published by other workers for senescent tissues, such as occur in ripening apples as described by Blackman (1928) and in other fruits (Hill, 1913).



## SUMMARY

Experimental data are given relating to the changes that occur in carbon dioxide production and in the values of the respiratory quotient, when germinating seeds of *Lathyrus odoratus*, *Fagopyrum esculentum*, and *Zea mais* are kept successively in air, nitrogen, and air.

Data obtained are discussed in relation to the possible light they throw on the nature of the respiratory processes of germinating seeds.

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