Dependence of mitosis and respiration in roots upon oxygen tension

BY J. E. AMOORE

Department of Botany, University of Edinburgh

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Excised pea-root tips were incubated for 4 h in gas mixtures containing 0-00001 to 100 % oxygen, in order to determine the effect upon mitosis. Below 0-0005 % oxygen, mitosis was completely arrested. Between 0-001 and 0-02 % oxygen, cells initially in mitosis completed division, but no more cells started dividing. Between 0-05 and 0-2 % oxygen, cells initially in interphase entered division, but did not finish. Above 0-5 % oxygen, all cells not prevented from dividing by excision finished division within 4 h. After exposure to 0-05 % oxygen for 4 h, an excessive proportion of cells was found in prophase; in 0-1 % oxygen an excess of metaphases, and in 0-2 % oxygen an excess of telophases resulted.

The oxygen uptake and carbon dioxide output of root tips were measured in a range of oxygen tensions and in anaerobic conditions. The relationship between oxygen uptake and oxygen tension was hyperbolic; a half maximum rate of oxygen uptake was obtained at about 10 % oxygen. It was concluded that the respiration of root tips was limited by slow diffusion of oxygen through the tissue. From the carbon dioxide output it was estimated that the amount of energy available to isolated root tips under anaerobic conditions was about 1 % of that available under aerobic conditions.

Possible mechanisms whereby extreme oxygen-lack could arrest mitosis were considered. It was shown that the arrest was not due to abolition of a gross supply of energy. No evidence was obtained as to what other mechanism might be operative. An hypothesis was formulated in an attempt to explain the complicated relationship between mitosis and oxygen tension. It was assumed that the visible phases of mitosis are immediately preceded by a phase with a higher requirement for oxygen than mitosis, and that preceding this is an earlier phase with a lower oxygen requirement than mitosis.

INTRODUCTION

In the preceding paper (Amoore 1961) it was shown that acute oxygen-lack or cyanide poisoning could arrest mitoses occurring in pea-root tips. However, if the conditions were not thoroughly anaerobic, mitoses in progress were able to continue slowly. The experiments described below were designed to investigate in detail the relationships between oxygen-tension, mitosis and respiration in excised pea-root tips.

METHODS

General methods

The general experimental methods were as previously described (Amoore 1961). Excised root tips from 48 h pea seedlings (Pisum sativum var. Meteor) were used throughout. In one experiment (see table 5) fifty-five root tips held in a perforated plate were cut simultaneously with a razor as described by Brown & Rickless (1949). For determining the mitotic index, use was frequently made of acetic-orcein squashes which had been prepared by squashing three similarly treated root tips together. This procedure combined the advantages of the quickness of a squash with the larger sample of a dispersion.
Experimental vessels

The 'closed' vessels (Amoore 1961) were used in one experiment, but were considered to be unsuitable for experiments with controlled low oxygen tensions, on account of the possibilities of gas exchange at the joints between rubber and glass, and of occlusion of air by the sintered-glass disk. So Warburg manometer vessels were used, which had the advantage of permitting measurements of oxygen uptake and carbon dioxide evolution by the direct method (Umbreit, Burris & Stauffer 1957). Usually 0·5 ml. of water was placed in the main well, and 0·2 ml. of N-potassium hydroxide in the centre well. In one experiment (table 2) no fluid was put in the main well, but 0·3 ml. of water was put in the side-arm to saturate the atmosphere with water vapour, and only 0·2 N-potassium hydroxide was used in the centre well, in order to avoid dehydrating the tissue. When low oxygen tensions were employed, all water used in the manometer vessels was previously freed of oxygen. The Brodie's fluid was earlier out-gassed in situ by suction.

For a still higher degree of anaerobiosis, the Warburg vessel was also considered to be unsatisfactory, because of the difficulty of filling its whole volume, including the manometer and connecting limb, with gas containing a low, controlled oxygen tension. So Thunberg tubes were used instead. Oxygen-free water (0·01 ml.) was placed in the bottom of the tube, and 0·04 ml. of water was put in the side-arm cap to saturate the atmosphere with water vapour. The Thunberg tubes were incubated in a water bath at 25° C.

Nitrogen

'Oxygen-free' nitrogen (containing less than 0·001 % oxygen) was supplied by the British Oxygen Company Ltd. It was used either without further treatment, or after scrubbing by passage through 250 ml. Dreschel bottles fitted with sintered-glass diffusers. For different experiments the nitrogen was scrubbed either with water alone, or with chromous chloride (two bottles) followed by water (Warburg, Kubowitz & Christian 1931), or with alkaline pyrogallol (two bottles) followed by water (Umbreit et al. 1957). As an additional precaution, when strictly anaerobic conditions were required, 0·3 ml. of chromous chloride was put in the side-arm of the Warburg vessel, or 2 ml. of alkaline pyrogallol were put in the side-arm cap of the Thunberg tube.

Preparation of mixtures of oxygen and nitrogen

Two methods were employed to obtain the required low oxygen tensions in the experimental vessels. The first is an indirect method but is applicable to a very wide range of oxygen tensions. The second, direct method, is perhaps more accurate, but only applicable to a narrow range of oxygen tensions.

(a) By repeated evacuation and refilling with nitrogen

The method was based on procedures described by Umbreit et al. (1957), with some modifications. In principle, suction was applied to reduce the pressure within the Warburg manometer vessel (or Thunberg tube) to $\frac{1}{3}$, $\frac{1}{4}$ or $\frac{1}{10}$ atmosphere. The vessel was then refilled with nitrogen. If the vessel initially contained air
Mitosis, respiration and oxygen tension

(20% oxygen), the resulting oxygen tensions would be 10, 5, or 2%. By repeating this process one or more times, the required oxygen tension was obtained. For example, a tension of 0.001% oxygen was obtained by evacuating once to \( \frac{1}{2} \) atmosphere and four times to \( \frac{1}{16} \) atmosphere, each time refilling the vessel with nitrogen. The apparatus and the procedure are described in figure 1.

Allowance was made for the water-vapour pressure within the flask as follows. If \( a \) was the barometric pressure and \( w \) was the water-vapour pressure, in mm mercury, then to obtain \( \frac{1}{x} \) atmosphere, evacuate to \( (a - w) - \left(\frac{(a - w)}{x}\right) \) mm mercury vacuum.

The dead-space in the manifold was minimized by treating one vessel at a time, by admitting the nitrogen as close to the vessel as possible, and by using narrow bore (capillary) tubing.

Both the suction and the nitrogen were controlled by means of pairs of screw-clamps acting on pressure tubing. One clamp of each pair was used as an adjustable leak, and the second clamp as a shut-off control. The setting of the leak on the suction side required adjustment according to the intended partial vacuum. Suction was provided by a filter pump, fitted with a trap to avoid suck-back. Nitrogen was supplied through a reducing valve and pressure regulator at about 20 cm water gauge. The water gauge allowed for up to 20 cm negative pressure when the nitrogen entered the partial vacuum in the flask.

For 100% oxygen, the manometer was flushed with oxygen. For 50% oxygen, it was first flushed with oxygen, then evacuated to \( \frac{1}{2} \) atmosphere and refilled with nitrogen.

When Thunberg tubes were treated, the tube which fitted the open limb of the Warburg manometer was closed with a clamp.

(b) By flushing with nitrogen and then adding air

The manometer vessels were first flushed with nitrogen for 5 min at about 200 ml./min, and allowed to equilibrate for 5 min in the thermostatic bath. Then air was injected through the side-arm stopper until the desired increase in pressure was registered by the manometer. The oxygen tension was one-fifth of the air pressure injected. The injector and procedure are described in figure 1. This method was limited to the range 0.02 to 0.5% oxygen.

The injector method was tested by means of alkaline pyrogallol in the manometer vessel, and found to give the required oxygen tension (0.1%) within about \( \pm 10\% \). Both injector and evacuation methods gave the same results within the range 0.02 to 0.5% oxygen, as well as could be judged by the effects on mitoses in pea-root tips.

The evacuation method was easier in practice, and was applicable to a far wider range of oxygen tensions, but any errors would be cumulative. The error in each cycle (due mainly to the partially compensating errors of dead-space and excess refilling pressure) should not exceed about \( \pm 5\% \). The nominal tensions of oxygen, shown in the tables and figures as percentages of an atmosphere, were those calculated from the filling procedures. No attempt was made to measure directly the actual oxygen tensions in the vessels.
FIGURE 1. Apparatus for obtaining mixtures of oxygen and nitrogen in Warburg manometer vessels. a is the Warburg manometer, b is the mercury manometer and c is the water-gauge. Suction was applied at d, and controlled by an adjustable leak e and shut-off control f. Nitrogen was led in at g, and controlled by a leak h and shut-off control j. The cycle of operations was as follows.

(i) Start with suction and nitrogen controls f and j closed, manometer tap open and Brodie's fluid lowered.

(ii) Adjust suction leak e for required vacuum, open suction control f, and allow mercury to rise to pre-determined level in manometer b. Close suction control.

(iii) Open nitrogen control j, and allow mercury to fall. Close nitrogen control. Raise and lower Brodie's fluid three times to mix gas in manometer with that in vessel. The cycle was repeated as often as necessary to obtain the required oxygen tension.

At the bottom left of the figure is the simple injector k used to add air to a manometer vessel previously flushed with nitrogen. The procedure was as follows.

(i) Start with vessel equilibrated at 25 °C, and with a small positive pressure in the vessel. Record reading in open limb of manometer. Attach injector, with end clamp l and oblique clamp m open, to sidearm stopper n of vessel.

(ii) Close end clamp l. Rotate stopper n to open. Simultaneously inject air with oblique clamp m to desired pressure, and adjust Brodie's fluid to keep level in closed limb of manometer at the mark. Rotate stopper to close.

(iii) Remove injector, shake manometer briefly, and read the pressure of air actually injected.
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Results

Effect of oxygen tension on mitosis in excised pea roots

A fixed incubation period of 4 h was selected for the following four experiments (tables 1 to 4). Each experiment contained three control samples. The first control (vessel 1) consisted of root tips fixed without incubation. The second control was root tips incubated for 4 h in air (20% oxygen). The third control was root tips incubated for 4 h in anaerobic conditions (0% oxygen). The remaining samples were incubated for 4 h in various mixtures of oxygen and nitrogen. Each oxygen tension was approximately one-half of the preceding tension. It transpired that a very wide range of oxygen tensions had to be covered (100 to 0.00001%). Accordingly four separate experiments were done, with some overlap of oxygen tensions between successive experiments. A graph showing the averaged data of tables 1 to 4 is given (figure 2).

Effects of oxygen tensions between 100 and 0.1% on mitosis

Exposure to oxygen tensions between 100 and 0.5% for 4 h permitted the mitotic index to decrease to one-third or less of the control value (table 1), with one exception. In 2% the fall in mitotic index was partially checked. Otherwise, any oxygen tension of 0.5% and above permitted most of the cells in mitosis to complete their division.

Table 1. Effect of oxygen tensions between 100 and 0.1% on mitosis in excised pea roots

Groups of 30 pea-root tips, 1.7 mm long, were put in Warburg manometer vessels containing 0.5 ml. of water in the main well, and 0.2 ml. of n-KOH in the centre well. Vessel 2 was flushed with oxygen, and vessel 12 with oxygen-free nitrogen, for 5 min at 400 ml./min. The other vessels were filled with the indicated mixtures of oxygen and nitrogen by the evacuation procedure described in Methods. The nitrogen was used without further treatment. Vessels 2 to 12 were incubated at 25°C for 4 h. The results of measurements of oxygen uptake are shown in figure 3. At the end of the incubation the tips were fixed with acetic-alcohol for subsequent observation of mitosis.

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In 0·2% oxygen, however, a high mitotic index was maintained. Also there was an unusual distribution of cells among the phases of mitosis, more than half the mitotic figures being telophases. The telophase count was more than double the control count at the start of the experiment (table 1, vessel 1). In 0·1% oxygen there was a higher mitotic index than was found in the control in air, and again there was an abnormal distribution among the phases of mitosis, for nearly two-thirds of all the mitotic figures were metaphases. The control in anaerobic conditions (table 1, vessel 12) showed some decrease in mitotic index, but it remained well above the control in air (vessel 4), and there was a normal distribution of phases.

**Effect of oxygen tensions between 0·4 and 0·025% on mitosis**

In this experiment (table 2, vessels 1 to 8) three samples (vessels 3 to 5) confirmed the results of the preceding experiment. An oxygen tension of 0·4% permitted cells in mitosis to finish dividing, but 0·2 and 0·1% oxygen both resulted in a small increase in mitotic index compared with the control (vessel 1). Again it was observed that 0·2% oxygen caused the appearance of an excessive proportion of cells in telophase, whereas 0·1% oxygen resulted in a very high metaphase count, nearly double the control.

**Table 2. Effect of oxygen tensions between 0·4 and 0·025%, and effect of 10−4 to 10−2 M-cyanide on mitosis**

Vessels 2 to 8 were Warburg manometer vessels containing 10 root tips each. No medium was put in the main well, but the side-arms held 0·3 ml. of water. The centre well received 0·2 ml. of 0·2N-KOH. Vessels 3 to 8 were flushed for 5 min at 200 ml./min with oxygen-free nitrogen which had been bubbled through water. Air was injected, as described in Methods, to give the indicated oxygen tension. Measurements of oxygen uptake will be described in a later section of the text.

Vessels 9 to 15 were the ‘closed’ vessels previously described (Amoore 1961). All vessels 2 to 15 were incubated at 25°C for 4 h.

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Mitosis, respiration and oxygen tension

An increased mitotic index was also observed in 0·05% oxygen. This oxygen tension produced a slightly elevated prophase count compared with the control at the start of the experiment. Prophases were much more numerous than other phases, but this is normal, owing to the greater duration of prophase compared with the other phases (Brown 1951). An excessive prophase count had been obtained several times when industrial nitrogen was used for 4 h (Amoore 1961); this agreed with the statement that the industrial nitrogen contained about 0·05% oxygen.

The mitotic index fell below the control when 0·025% oxygen was present during the incubation. In the intended anaerobic vessel (table 2, vessel 8) the mitotic index fell to a very low value. This was probably on account of a trace of oxygen (less than 0·025%) remaining in the vessel. The vessel was flushed for 5 min at only 200 ml./min, compared with 400 ml./min used for vessel 12 in table 1. Evidently the higher rate of passing oxygen-free nitrogen is necessary to lower the oxygen tension in a manometer vessel sufficiently to arrest mitosis.

The same experiment included additional manometer vessels (not shown in table 2), which indicated that the effect of oxygen tension on mitosis was the same, whether the root tips were covered with water, or kept in moist gas.

Effect of $10^{-4}$ to $10^{-2}$ m-cyanide on mitosis

The experiment in table 2 gives a comparison of the effects on mitosis of a series of concentrations of cyanide (vessels 9 to 15) with the effects of a series of tensions of oxygen. Whereas $10^{-4}$ m-cyanide did not affect the normal decrease in mitotic index which occurred in air, higher concentrations caused progressively more complete arrest of mitosis. Cyanide at $2 \times 10^{-3}$ m was approximately as effective as 0·1% oxygen in arresting mitosis, so far as could be judged by the effect on mitotic index. Both conditions produced elevated counts of metaphases and telophases. Progressive doubling of cyanide concentration was not so effective as progressive halving of oxygen tension. Cyanide did not appear to be as selective as oxygen-lack in causing a preponderance of individual phases of mitosis.

Effect of oxygen tensions between 0·1 and 0·001% on mitosis

As before, in this experiment three samples (table 3, vessels 3 to 5) confirmed the results of the preceding experiment. After 4 h in 0·1% oxygen more cells were in metaphase than in any other phase, and in 0·05% oxygen the majority were in prophase. The prophase count was higher than the initial control. Again, in 0·02% oxygen there was a decreased mitotic index compared with that in 0·05% oxygen.

When the oxygen tension was lowered to 0·01% a higher mitotic index was obtained, but progressive halving of the oxygen tension to 0·005, 0·002 or 0·001%, all resulted in a low mitotic index. The index actually fell to about one-third of the control, so the results of incubating for 4 h in oxygen tensions from 0·005 to 0·001% were indistinguishable from the result of a corresponding incubation in air.

However, in vessel 10, which was kept thoroughly anaerobic by the presence of chromous chloride in the side-arm, a substantial degree of mitotic arrest was
achieved. Therefore an oxygen tension of less than 0·001 % was required to arrest mitosis. Additional vessels (not included in table 3) showed that it was not necessary to scrub the oxygen-free nitrogen with chromous chloride, or to put chromous

TABLE 3. EFFECT OF OXYGEN TENSIONS BETWEEN 0·1 AND 0·001 % ON MITOSIS

Vessels 2 to 10 were Warburg manometer vessels containing 0·5 ml. of water in the main well, but no KOH in the centre well. The indicated oxygen tensions were established in vessels 3 to 9 by the evacuation method. Vessel 10 was flushed with oxygen-free nitrogen for 5 min at 400 ml./min, and also contained 0·3 ml. of m-CrCl₃ in the side-arm. The nitrogen was scrubbed with CrCl₃. No manometric observations were made on these vessels.

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<th>vessel no.</th>
<th>oxygen tension (%)</th>
<th>time (h)</th>
<th>prophase (%)</th>
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TABLE 4. EFFECT OF OXYGEN TENSIONS BETWEEN 0·02 AND 0·0001 % ON MITOSIS

Vessels 2 to 14 were Thunberg tubes containing 0·01 ml. of water and 5 pea-root tips. The indicated oxygen tensions were established in vessels 3 to 13 by the evacuation method. Vessel 14 was treated like vessel 13, but also received 2 ml. of alkaline pyrogallol in the side-arm cap. The nitrogen was scrubbed with alkaline pyrogallol.

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</table>
Mitosis, respiration and oxygen tension

chloride in the side-arm. Flushing the manometer vessel for 5 min with oxygen-free nitrogen direct from the cylinder was adequate to arrest mitosis, provided the rate of passage of the gas was at least 400 mL/min. This provided indirect evidence that the oxygen-free nitrogen contained less than 0·001% oxygen. It was also found that the presence or absence of potassium hydroxide in the centre well had no effect on the mitotic index in air or in nitrogen.

Effect of oxygen tensions between 0·02 and 0·00001% on mitosis

In this experiment very low oxygen tensions were required, so Thunberg tubes were employed. The results are shown in table 4. Vessels 3 to 7 of table 4 duplicated the oxygen tensions in vessels 5 to 9 of table 3, and confirmed the results. A uniformly low mitotic index was observed at all oxygen tensions from 0·02 to 0·001%, except for 0·01%, which resulted in a high mitotic index.

All oxygen tensions from 0·0005 down to 0·00001% produced almost complete arrest of mitosis, which was indistinguishable from the arrest obtained in the anaerobic vessel containing alkaline pyrogallol in the side-arm cap (vessel 14). There were no consistent alterations in the distribution of cells among the phases of mitosis, compared with the control. Therefore, during a period of 4 h, mitosis was completely arrested, provided the oxygen tension was not greater than 0·0005%.

Summary of effects of oxygen tensions between 0·00001 and 100% on mitosis

The data in tables 1 to 4 were used to construct the curves shown in figure 2, which serves to summarize the complicated results of the preceding four experiments. The curves represent the prevalence of each phase of mitosis, after incubating excised root tips for 4 h at 25 °C, in the presence of a wide range of oxygen tensions.

The salient points are as follows. Up to 0·0005% oxygen, the mitotic index remained high, slightly below the control value. This must represent almost complete arrest of mitosis. Between 0·001 and 0·02% oxygen a low mitotic index resulted. Evidently enough oxygen was present to permit many cells which were in mitosis at the start of the experiment to continue and complete their division, but no more cells were able to start dividing. Between 0·05 and 0·2% oxygen there was again a high mitotic index. This probably represents cells which were in interphase at the start of the experiment, and which were able, in the presence of these oxygen tensions, to enter mitosis, but not enough oxygen was available for them to finish division within 4 h. However, in oxygen tensions of 0·5% and above, a low mitotic index was observed. Presumably ample oxygen was present for all cells capable of dividing to start and finish division within 4 h.

The above description applies only to the major features of the mitotic index curve. It was an irregular curve, and minor peaks occurred at 0·01 and at 2% oxygen. Part of the irregularity of the mitotic index curve was due to its being composed of four separate curves representing the phases of mitosis. These curves (figure 2) were somewhat simpler, but the peaks in each curve occurred at increasing oxygen tensions. Thus a peak was found in the prophase curve at 0·05% oxygen,
in the metaphase curve at 0·1% oxygen, in the anaphase curve at 0·05–0·1% oxygen, and in the telophase curve at 0·2% oxygen. In each curve the peak value was appreciably above the control value (horizontal broken line). It is also noticeable that with increasing oxygen tension, the telophase count began to decrease at a lower oxygen tension (0·0002%) than did the metaphase count (0·0005%) or the prophase count (0·001%). Each curve also contained other irregularities not mentioned in the above description.

**Figure 2.** Effect of oxygen tensions between 0·00001 and 100% on mitosis in excised pea-root tips. The graphs were based on the data in tables 1 to 4. Means were taken when 2 or more experiments were done at the same oxygen tension. The experiments lasted for 4 h at 25 °C. The horizontal broken lines represent the average percentage of meristic cells in each phase of mitosis at the start of the experiments.

**Effect on mitosis of delay (0 to 3 h) between excising root tips and exposing them to reduced oxygen tensions (0·2, 0·1 and 0·05%)**

In this experiment a number of root tips were cut simultaneously, instead of singly as in earlier experiments. The tips were incubated in air for 7 h, and samples were fixed every hour. The changes in mitotic index are shown in table 5. The
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mitotic index remained high for up to 3 h after excising the tips. Then it decreased, as usual. These observations suggest that cell division continued for some time after excising the tip. Thus, any cells which would naturally have started mitosis in 3 h or less were unaffected by excision, and were able to start and finish their divisions normally.

Table 5. Effect on mitosis of delay (0 to 3 h) between excising root tips and exposing them to reduced oxygen tensions (0.2, 0.1 and 0.05 %)

All root tips used in this experiment were cut simultaneously, then kept on a wetted sintered glass disk in a Petri dish at 25 °C until transferred to Thumberg tubes containing 0.01 ml. of water. The evacuation method was used to provide the indicated oxygen tensions. Oxygen-free nitrogen was used without further treatment.

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In the experiments described in this and the preceding paper (Amoore 1961), there had been a variable delay of up to 3 h between excising an individual tip, and placing it in the chosen gas mixture. This delay arose from the time required to excise up to 300 tips, and to prepare up to fifteen different gas mixtures. In the present experiment (table 5), a simplified test, requiring only three gas mixtures and nine tips, was repeated at hourly intervals after cutting a large group of tips simultaneously. The incubation period was 4 h, and the oxygen tensions were 0.2, 0.1 and 0.05 %. In earlier experiments these conditions resulted in an excessive proportion of cells appearing in telophase, in metaphase and in prophase, respectively.

It was found, however, that the above characteristic distribution of cells among the phases of mitosis only developed in root tips which had been kept in air for
2 or 3 h, before being placed in reduced oxygen tensions for 4 h (table 5, vessels 15 to 20). Tips which were kept in air for only 1 h before being placed in low oxygen tensions developed a high telophase count in 0·2 % oxygen, but no high metaphase or prophase counts were found in 0·1 or 0·05 % oxygen (table 5, vessels 12 to 14). Tips which were placed in low oxygen tensions directly after excision (9 to 15 min delay) showed a completely different result (table 5, vessels 9 to 11). A high prophase count was obtained in 0·2 % oxygen, and a high telophase count in 0·05 % oxygen, which was the reverse of the earlier findings. Nevertheless, irrespective of the duration of the delay (0 to 3 h) a higher mitotic index was maintained after 4 h in the presence of low oxygen tensions than after a corresponding period in air. Only the distribution of cells among the phases of mitosis was altered by the delay.

It may be concluded from this experiment that the results shown in tables 1 to 4 and in figure 2 are characteristic only of root tips which had been excised 2 to 3 h before being placed in different gas mixtures.

![Figure 3](http://rspb.royalsocietypublishing.org/)

**Figure 3.** Effect of oxygen tension on respiration in excised pea-root tips. The measurements were made with Warburg manometers which were set up as described in table 1. The lowest point on the graph represents 0·2 % oxygen. No uptake of oxygen was detected in 0·1 % oxygen or in the oxygen-free nitrogen.

**Effect of oxygen tension on oxygen uptake by excised pea-root tips**

In conjunction with the experiment shown in table 1, measurements were made of the oxygen uptake by excised pea-root tips in a range of oxygen tensions from 100 to 0·1 %. Each manometer vessel contained 0·5 ml. of water and 30 tips, 1·7 mm long. The rate of oxygen uptake was nearly constant during 4 h at all oxygen tensions. The rate of oxygen consumption was expressed as the metabolic quotient, $Q_{O_2}$, which represents microlitres of oxygen absorbed per milligram of dry weight per hour. The results are shown in figure 3. The $Q_{O_2}$ in air (20 % oxygen) was $-7·8$. To achieve the maximum rate of oxygen uptake ($Q_{O_2}$ of $-9·4$) it was necessary to employ an oxygen tension substantially greater than that in air, perhaps 40 to 50 % oxygen.
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The curve relating $Q_{O_2}$ to oxygen tension was hyperbolic, i.e. a reciprocal plot of $1/Q_{O_2}$ against $1/O_2$ was approximately linear. From the reciprocal plot it appeared that in 0·1% oxygen the $Q_{O_2}$ would have decreased to approximately 1% of its maximum value. However, below 1% oxygen the rate of oxygen uptake was too low for accurate measurement, and no oxygen uptake could be detected in 0·1% oxygen.

![Graph showing gas exchange over time](image)

**Figure 4.** Respiration of pea-root tips. The curves represent the respiration of groups of 30 tips, 1·7 mm long. ○, oxygen uptake in air; ●, carbon dioxide evolution in air; +, carbon dioxide evolution in nitrogen.

In the experiment in table 2, the manometer vessels contained no medium in the main well, but had 0·3 ml. of water in the side-arm to keep the gas moist. Additional vessels were set up, containing 3 ml. of water in the main well, but otherwise similar. It was found that in 20% oxygen and in 0·2% oxygen, the presence of water had no effect on the oxygen uptake. Evidently the oxygen uptake of pea-root tips is unaffected by immersion in well-agitated water.

After incubation for 4 h in 0·2% oxygen, the manometer vessel was flushed with air. Oxygen uptake began and continued for at least 3 h, but only at about one-third of the initial rate of oxygen uptake by root tips in air. However, the oxygen uptake by tips which had been in air throughout had decreased between 4 and 7 h to about two-thirds of the initial rate.

$Q_{O_2}^\text{air}$, $Q_{CO_2}^\text{air}$ and $Q_{CO_2}^N$ of pea-root tips

The respiration of groups of thirty root tips was measured aerobically and anaerobically for 4 h. The results are given in figure 4, which shows the running totals of gas exchanges. For the first hour, the aerobic oxygen uptake was almost equalled by the aerobic carbon dioxide output. During this period the $Q_{O_2}^\text{air}$ was $-9·6$, $Q_{CO_2}^\text{air}$ was $+9·4$, and r.q. was 0·98. However, an inflexion occurred in both
curves at 1 h, and furthermore the curves diverged. For the remaining 3 h, the $Q_{\text{air}}^{\text{air}}$ was $-8.0$, $Q_{\text{CO}_2}^{\text{air}}$ was $+6.7$, and r.q. was $0.84$.

During the first hour the anaerobic carbon dioxide output was $48\%$ of the aerobic ($Q_{\text{CO}_2}^{\text{air}}$ was $+4.5$). However, after 1 h there was a sharp inflexion in the curve, and the $Q_{\text{CO}_2}^{\text{air}}$ decreased to only $+0.5$. The anaerobic carbon dioxide output during the last 3 h was only $8\%$ of the aerobic.

**DISCUSSION**

*Respiration of excised root tips*

The curve showing the dependence of oxygen uptake upon oxygen tension was hyperbolic (figure 3). Similar hyperbolae have been obtained for onion bulb roots (Berry 1949; Rosene 1950), and for rice and barley seedlings (Vlamis & Davis 1943). In the present work, it was found that the oxygen tension in air was a limiting factor for the respiration of pea-root tips; an oxygen tension of 40 to 50\% was necessary for a maximum rate of oxygen uptake. Onion roots also required a higher oxygen tension than that in air to achieve maximum respiration (Berry 1949; Rosene 1950).

Considering the great affinity of the isolated respiratory enzymes for oxygen, it is remarkable how high an oxygen tension is necessary for an intact root tip to respire freely. The oxygen tension for a half maximum rate of oxygen uptake by excised pea-root tips was about $10\%$ oxygen. Chance (1957) showed that the respiratory particles from heart muscle reached half the maximum rate with $\mu$m-oxygen in the medium (equivalent to only about $0.1\%$ oxygen in the gas phase). Certain plant tissues show a comparable affinity for oxygen, but only under particular conditions of measurement. Thus Thimann, Yocum & Hackett (1954) found that potato tuber slices reached half maximum respiration in about $0.1\%$ oxygen, provided the slices were thin ($0.5$ mm), the temperature was low ($15$ °C), and the respiration was measured in a moist gas phase. Yocum & Hackett (1957) showed that slices of aroid spadix reached half maximum respiration in $0.2\%$ oxygen, provided that the respiration was measured in moist gas; if the tissue was immersed in fluid in a Warburg manometer vessel, the half maximum respiration was only reached at about $16\%$ oxygen. Ohmura & Howell (1960) also found an inhibitory effect of water on the respiration of many different plant tissues. They presented evidence which suggested that the inhibition was due to the presence of water in the normally air-filled intercellular spaces of the tissue, thereby greatly slowing down the penetration of oxygen to the cells.

Berry (1949) showed that there are no intercellular air spaces in the meristematic region of the root tip. Therefore respiration must be limited by the slow diffusion of oxygen through the liquid phase. From his measurements he calculated the apparent diffusion coefficient of oxygen through onion root-tip tissue. The value was about one-quarter of the diffusion coefficient of oxygen through water. When a similar calculation was applied to the present data on pea-root tips, the diffusion coefficient appeared to be approximately one-half of the value for water. It may be concluded that the low affinity of root tips for oxygen must be due to the slow
diffusion of oxygen through the tissue. The observation in the present work that the respiration of root tips was unaffected by the presence of well-agitated water is in accordance with this conclusion, because there are no air spaces to become waterlogged. Furthermore, as pointed out by Read (1952), the concentration of oxygen in the interior of the root may be considerably less than that in the surrounding medium.

During the first hour of incubation (figure 4) the anaerobic carbon dioxide production by pea-root tips was about 48% of the aerobic. Vlamis & Davis (1943) found that during 12 h in 0·2% oxygen the carbon dioxide output by rice and barley seedlings was 52% of that in air. In the present work, the anaerobic carbon dioxide output decreased sharply after 1 h to about 8% of the aerobic. This may have been due to lack of fermentable substrate, for none was present in the medium, and the tips were isolated from the seedlings. Possibly a similar explanation applies to the change in respiratory rate and respiratory quotient which also occurred after 1 h incubation. These measurements of respiratory exchanges permitted an approximate estimate to be made of the relative amounts of energy available to the isolated root tips under aerobic and anaerobic conditions. It was assumed that each mole of carbon dioxide evolved anaerobically represented only one-sixth of the energy produced per mole evolved aerobically (Baldwin 1952). During the first hour, the energy yield in anaerobic conditions would be about 10% of that in aerobic conditions, and subsequently it would fall to about 1%.

Mechanism of mitotic arrest by oxygen-lack

There are at least five possible mechanisms which might serve to explain why an extreme lack of oxygen can arrest mitoses in progress. Only regarding the first mechanism does the present work provide any evidence, and that tends to be unfavourable. No attempt will be made to choose among the remaining four mechanisms.

Abolition of gross supply of energy

Bullough (1952) put forward the view that the energy necessary for mitosis is built up by normal metabolism (respiration or glycolysis) during interphase, and that once a mitosis begins it continues without requiring any additional energy. This view, which has been repeated by Swann (1957) and by Stern (1959), is based on the observation that, in tissues which have been poisoned with respiratory or glycolytic inhibitors, any cells which were in mitosis continue their division, but no more cells enter mitosis. If this argument is applied to the present results, the conclusion would be that because acute oxygen-lack or cyanide poisoning arrests cells in mitosis, it follows that a continuous supply of energy is also required during mitosis, as well as in interphase.

Although such a conclusion would be in qualitative agreement with the present findings, it does not seem likely on quantitative grounds. It was shown above that under anaerobic conditions, the energy available to isolated root tips by glycolysis would be only about 1% of that available under aerobic conditions by respiration. Furthermore, extrapolation of the reciprocal plot of the hyperbola shown in figure 3
indicated that the energy available by respiration would have decreased to about 1% of normal in 0.1% oxygen. Yet to arrest mitosis completely for 4 h demanded a reduction of oxygen tension to 0.0005% oxygen (figure 2). Further extrapolation indicated that respiration at this oxygen tension would have decreased to about 0.005% of normal. Now, at all oxygen tensions below 0.1% oxygen, the total energy available from respiration plus glycolysis must have been substantially constant at about 1% of normal. Therefore the arrest of mitosis by extreme oxygen-lack cannot be due to the abolition of the gross supply of energy.

It may be noted that mitoses in progress were able to continue when the total production of energy was decreased by low oxygen tensions to 1% of the normal aerobic value. Bullough (1952), Swann (1957) and Gelfant (1959) pointed out that virtually any reduction below 100% in the respiration of normally aerobic tissues (mouse-ear epidermis or sea-urchin egg) results in a reduction in the frequency of cell division. This may also be true of pea roots, for if the aeration of intact seedlings was at all inadequate, the mitotic index decreased (Amoore 1961). Therefore the continuation of mitoses in progress apparently requires not more than 1% of the energy required by cells to enter division. The conclusions of Bullough (1952), of Swann (1957) and of Stern (1959) are therefore supported, that the energy requirements of mitoses are largely anticipated before cells enter prophase. However, the experimental data which formed the basis for their conclusions may require some extension, for the present work showed that extreme oxygen-lack or cyanide poisoning can arrest mitoses in progress.

**Abolition of minute supply of aerobic energy**

The observed dependence of mitosis upon a trace of oxygen might be due to the necessity for a minute amount of energy which must obligatorily be supplied by aerobic metabolism. Thus Swann (1953) suggested that a mitotic controlling substance might have to be synthesized aerobically, and that if this was available, the energy for division could be obtained anaerobically.

**Solution of mitotic apparatus**

A trace of oxygen might be necessary for the coherence of the mitotic apparatus (asters, astral rays and spindle). Harvey (1927) found that withdrawal of oxygen from dividing sea-urchin eggs caused the astral rays and mitotic figure to disappear, but they reappeared in the same position and phase on re-admitting oxygen.

**Adhesion of chromosomes**

Steinjitz (1943) showed that oxygen-lack caused the appearance of abnormal mitoses, owing to the development of sticky chromosome bridges. If this stickiness became intense, it might arrest mitosis completely.

**Interference with binding of chromosomal material by iron**

Kihlman (1957) and Kihlman, Merz & Swanson (1957) have suggested that iron is important as a structurally linking material in chromosomes, and that its state of oxidation and co-ordination determines the lability of chromosomes to radiation.
and other interference. In oxygen deficiency the state of the iron might be altered so that the re-organization of chromosomal material during mitosis could no longer occur.

Hypothesis for abnormal phase distribution

In the Discussion of the preceding paper (Amoore 1961) a simple hypothesis was advanced to account for the main observations. It was that all stages of cell division depend upon the presence of oxygen, but the visible phases of mitosis are less dependent than is the stage of entering mitosis. However, it was noted that several observations (irregular mitotic index, abnormal distributions among the phases of mitosis, and elevation of counts of individual phases) would not fit into this hypothesis. The present work (tables 1 to 4 and figure 2) showed that the origin and controlling factor of these variations was the exact value of the oxygen tension present during the experiment. Nevertheless, the problem arises of how a single variable, oxygen tension, produces such a variety of effects on mitosis. The following is an attempt to formulate an hypothesis to account for these variations.

In figure 2, oxygen tension was the independent variable, and a fixed time of 4 h was selected. It was found that oxygen tensions of 0·001 to 0·02% permitted mitoses in progress to continue, but a considerably higher oxygen tension of 0·05 to 0·2% was necessary for cells initially in interphase to enter mitosis. This suggests that, immediately prior to the visible prophase of mitosis, there is a phase with a much higher oxygen requirement than that of mitosis itself. Now, oxygen tensions between 0·05 and 0·2% caused the development of higher counts of individual phases of mitosis than were initially present. This implies that, sometime before the visible phases of mitosis, there is a phase with an even lower oxygen requirement than that during mitosis. Finally, at oxygen tensions above 0·5%, all cells capable of doing so finished dividing within 4 h. No more cells were able to enter division, because they were cut off from the seedling.

In the above experiments, the oxygen tension was varied, with a fixed time of 4 h. It is probable that similar results would have been obtained by varying the time, with a fixed oxygen tension of, for example, 0·05%. The above considerations may be expressed diagrammatically more easily if time, instead of oxygen tension, is the independent variable. This has been done in figure 5. Molé-Bajer (1955) used a diagram of this type to represent the progress of cells through mitosis under different conditions.

Zero time on the x axis represents the time when the excised root tips were transferred from air to an atmosphere containing only 0·05% oxygen. The y axis represents, on the same scale, the progress of cells through the phases of mitosis (development). Zero time on the y axis represents the start of visible prophase. Prophase lasts for about 1 h, and metaphase and telophase for about ½ h each, in pea roots at 25 °C (Brown 1951). Before zero time on the y axis, and after 1½ h, the cells are in interphase.

In the intact seedling, cells are randomly distributed throughout all phases of the division cycle. This has been indicated by placing dots, representing cells, at equal intervals (½ h) along the y axis. During the first hour shown in the figure (−3 to −2 h), each cell progresses 1 h through its normal development, which is
represented by a straight line with slope of unity (1 in 1). At \(-2\) h on the \(x\) axis, the root tips were excised, but kept in air. The effect of excision is to prevent cells which were more than 3 h from their natural entry into mitosis from dividing at all (table 5). The development of such cells is assumed to have stopped altogether, so their progress curves become horizontal (slope of 1 in \(\infty\)).

![Diagram](http://rspb.royalsocietypublishing.org/)

**Figure 5.** Diagram representing the relation between mitotic development and time, according to a hypothesis described in the text. The development is divided into interphase (I), prophase (P), metaphase (M), anaphase (A) and telophase (T). At the start of the experiment cells (dots) were spaced at equal intervals along the development axis. The hypothetical susceptibilities of different phases of development to oxygen lack are indicated at the right of the figure. At \(-2\) h the root tips were excised, and at zero time they were placed in 0.05% oxygen. These actions would alter the slope of the progress curves, and hence the proportion of cells in each phase of mitosis, as indicated in the figure.

At zero time (2 h after excision) the tips were placed in an atmosphere of 0.05% oxygen. As a working hypothesis, it is postulated that the susceptibility of the cells to oxygen-lack depends upon their degree of development, that is, their position in the division cycle. Suppose that cells in the visible phases of mitosis, or in very early interphase, are slowed to one-half their normal rate of development by 0.05% oxygen. Then the slope of their progress curves becomes 1 in 2. Just prior to the onset of visible prophase, suppose there is a period of \(\frac{1}{2}\) h during which exposure to 0.05% oxygen slows their development to one-fifth the normal rate. The slope of the progress curve becomes 1 in 5. Finally, suppose that cells which were in late interphase (between 3 and \(\frac{1}{2}\) h before they would normally enter prophase) are unaffected by an atmosphere of 0.05% oxygen. The slope of their progress curves remains unaltered at 1 in 1.
Mitosis, respiration and oxygen tension

The consequences of such an hypothesis may be seen by following the progress of cells in each phase of development, with the appropriate alterations of slope on passing from one degree of susceptibility to another (figure 5). During the first 2½ h after placing in 0·05% oxygen there would be a fall in mitotic index, represented by a decreased density of cells (progress curves) in the visible phases of mitosis. Then would follow a period of about 2 h with a high prophase count (equal to the control count at the start of the experiment), represented by an increased density of cells in prophase. This would be followed ½ h later by an elevated metaphase count, and ¾ h after that by an abnormally high telophase count. Accompanying each unusually high count of individual phases would be a low count of the other phases. Finally, 6 h after placing in 0·05% oxygen the mitotic index would fall to a very low level.

This hypothesis can account for nearly all the observations reported in the present and the preceding paper (Amoore 1961). Cells partially arrested by low oxygen tensions do eventually complete mitosis and form two new cells (Amoore 1961, figures 2 and 4). An elevated prophase count was preceded by a low mitotic index, and followed ½ h later by an elevated metaphase count (Amoore 1961, figure 4). After a partial arrest of mitosis the mitotic index falls to a very low value (Amoore 1961, figure 3).

If it is further assumed that an increase in oxygen tension increases the slope of the progress curves (figure 5), then the complicated curves in figure 2 receive an explanation. At all oxygen tensions below 0·001% development ceased, the progress curves became horizontal, and a high mitotic index was maintained for 4 h. Between 0·001 and 0·02% oxygen, enough progress would be made by cells in mitosis to reach interphase, but few cells would have been able to enter mitosis, resulting in a low mitotic index. With a slightly higher oxygen tension, 0·05%, and a steeper slope, a large number of partially arrested cells would have reached prophase, giving an elevated prophase count at 4 h (figure 5). Progressively steeper slopes, permitted by 0·1% and by 0·2% oxygen, would result in elevated metaphase and telophase counts respectively after 4 h. Above 0·5% oxygen there can have been little if any lessening of the slope below its normal 1 in 1, so all cells not prevented from doing so by excision were able to complete division within the 4 h limit.

It should be noted that the degrees and durations of inhibitions by 0·05% oxygen were arbitrarily selected in the above hypothesis, in order to give as good agreement as possible with the observations. The solution presented above is not unique, for a permutation of appropriate times and degrees of inhibition could produce similar effects on counts of cells in different phases of mitosis. Furthermore, there are still several results which even this more elaborate hypothesis will not explain. For instance, at oxygen tensions below 0·001%, the telophase count began to decrease before the prophase count (figure 2), whereas according to the hypothesis in figure 5 the prophase count should fall first. Also, the effect of delay between excising and placing the tips in low oxygen tensions (table 5) does not receive a complete explanation. The minor peaks at 0·01 and 2% oxygen in the mitotic index curve (figure 2) are also unexplained. However, it is felt that an
hypothesis which makes some contribution towards explaining the complicated
effects of oxygen-lack upon mitosis is worth while.

There is some evidence, from measurements of the respiration of developing
anthers, that the oxygen requirement of the cell fluctuates during the division
cycle. Erickson (1947) found that in the anthers of *Lilium longiflorum* there were
two distinct falls in the respiration below the general level, corresponding with
meiosis and with the subsequent mitosis. Stern & Kirk (1948), using anthers of
*Trillium erectum*, found that the oxygen uptake rose irregularly during the pre-
mitotic stages, but immediately preceding and during division there was a sharp
fall in oxygen consumption. These findings are in general agreement with the
postulates of the above hypothesis.

*Re-appraisal of published observations*

If the relationships between oxygen tension, mitosis and respiration are as com-
licated in other tissues as they are in pea roots (figure 2), it will account for the
confusing and contradictory nature of many published observations, particularly
if one considers the minuteness of the oxygen tension which permits mitosis to
continue. In pea roots, the limiting tension to arrest mitoses for at least 4 h was
0.0005%. To achieve such a low oxygen tension requires gas-tight apparatus, a
supply of oxygen-free gas, and the use of oxygen-free media, perhaps supplemented
by including an oxygen-absorbing substance in the vessel. In assessing published
work, it is not always possible to ascertain how thorough was the exclusion of
oxygen. However, there are some indications that if a high degree of anaerobiosis
was achieved, then mitoses in progress were arrested.

Thus in the experiments of Nabokich (1904) and of Steinitz (1943) mitosis in
roots was very probably arrested, even though the authors did not place this
interpretation upon their findings (Amoore 1961). Nabokich used yellow phos-
phorus and Steinitz used alkaline pyrogallol to maintain anaerobic conditions.
Harvey (1927) used hydrogen freed of oxygen by passage over red-hot platinized
asbestos, and showed that mitosis in the sea-urchin egg could be stopped at any
phase. Ephrusi, Chevillard, Mayer & Plantefol (1929) freed the nitrogen from
oxygen by treatment with alkaline pyrogallol and yellow phosphorus, and further-
more they analysed their gas mixtures eudiometrically. They found that, below 1%
oxygen, division ceased in prophase in cultures of chick-heart fibroblasts. Havard
& Kendal (1934) purified nitrogen with chromous chloride, and showed that cell
division ceased in cultures of embryonic chick-heart. Bajer (1954) noted that if
oxygen supply was poor, endosperm cells in culture were liable to die in prophase.

Conversely, experiments in which mitoses in progress were reported to continue,
do not appear to have been so satisfactory as regards establishing anaerobiosis.
Demoor (1895) found that mitoses in staminal hairs of *Tradescantia* continued to
telophase, but then stopped, in a stream of unpurified hydrogen or in a partial
vacuum (7 or 8 cm Hg). However, neither of these conditions would give good
anaerobiosis. Bullough & Johnson (1951) incubated mouse-ear epidermis in
saline in Warburg vessels, and found that if the gas phase was nitrogen the number
of cells in mitosis rapidly decreased. However, apart from any question of the
purity of the nitrogen, if the saline was not first freed of oxygen, calculation suggests that it might have contributed enough oxygen to allow mitoses in progress to continue. Gelfant (1959) withheld any evaluation of mitotic activity in epidermal cells cultured in nitrogen, on account of the resulting necrosis. Harris (1956) relied upon the respiration of rat connective-tissue cells in culture to lower the oxygen tension, and noted that multiplication of cells continued after measurable respiration had ceased. However, respiration apparently ceased with about 5 \( \mu \text{atm} \) oxygen remaining in the medium. This is equivalent to 0.5% oxygen in the gas phase, so it is unlikely that respiration could lower the oxygen tension sufficiently to arrest mitoses in progress. Swann (1953) used 97% carbon monoxide plus 0.6% oxygen to inhibit respiration in sea-urchin eggs, and found that mitoses in progress would continue. However, carbon monoxide at less than 1 atmosphere might not have suppressed oxygen uptake completely. It is interesting to note that if the inhibition was applied after the start of mitosis, metaphase and anaphase were not delayed, but prophase was slowed.

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