

OXYGEN TENSION AND THE RATES OF MITOSIS AND INTERPHASE IN ROOTS

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ABSTRACT

The object of this work was to determine the influence of a wide range of oxygen tensions upon the relative rates of respiration, mitosis, and interphase in pea root tips, compared with the normal rates of these processes in air. From the rates of disappearance of mitotic figures in excised tips kept in various oxygen tensions, the relative rates of mitosis were found to decrease gradually from 122 per cent in 100 per cent oxygen to 24 per cent in 0.0007 per cent oxygen. From the mitotic indices of intact seedlings, the relative rates of interphase were found to decrease sharply from 82 per cent in 10 per cent oxygen to 6 per cent in 5 per cent oxygen. The data on relative rates of respiration, mitosis, and interphase in root tips were compared, and it was shown that the three processes are perfectly distinct in their quantitative relationships to low oxygen tensions.

The cells of most tissues require a normal supply of oxygen if they are going to start dividing. However, once the cells have reached the visible stages of division, or mitosis, they are able to continue the process and form two new cells, even in the apparent absence of oxygen (Bullough, 1952; Swann, 1957; Stern, 1959). Whether mitosis can continue in the complete absence of oxygen has been a matter for controversy. Recently it has been demonstrated that in pea-root tips, mitoses occurring in the meristematic cells can definitely be arrested, at any phase, by the complete removal of oxygen (Amoores, 1961 *a*). The explanation of earlier conflicting evidence over this point probably lies in the remarkably low oxygen tension (less than 0.0005 per cent) which must be maintained if mitoses in progress are to be almost completely arrested (Amoores, 1961 *b*).

In the previous investigation (Amoores, 1961 *b*) it was necessary to use qualitative terms like "complete arrest," "partial arrest," and "slowly continues," in describing the effects on mitosis and interphase. This is because the data consisted

of cytological counts of the mitotic index in the fixed material. Now the mitotic index by itself yields only the relative durations of each phase of the division cycle, but gives no indication of the actual rate of mitosis. Furthermore, Amoores (1961 *b*) showed that when mitosis was partially inhibited, the mitotic index became a very elaborate function of the duration and degree of inhibition. Yet if meaningful comparisons are to be made between respiration, mitosis, and interphase, it is necessary to express all three, quantitatively, in terms of rates.

The object of the work described in this paper was to show that the relative rates of mitosis in various oxygen tensions may be deduced from observations of the mitotic indices in excised pea root tips, at known intervals of time or oxygen tension. From observations of the mitotic index in the root tips of intact seedlings kept in a range of oxygen tensions, it was possible also to determine the relative rates of interphase.

The results were used to plot the relative rates of respiration, mitosis, and interphase, as functions

of oxygen tension. The curves show that the three processes have quite different dependencies upon oxygen tension.

METHODS

EXPERIMENTS ON ROOT TIPS: Excised root tips (1.7 mm in length) from 48-hour pea seedlings (*Pisum sativum*, var. Meteor) were used. Three root tips were placed in each vessel. The general experimental methods were as described previously (Amoore, 1961 *a, b*). In all experiments the samples were incubated at 25°C, usually in the dark.

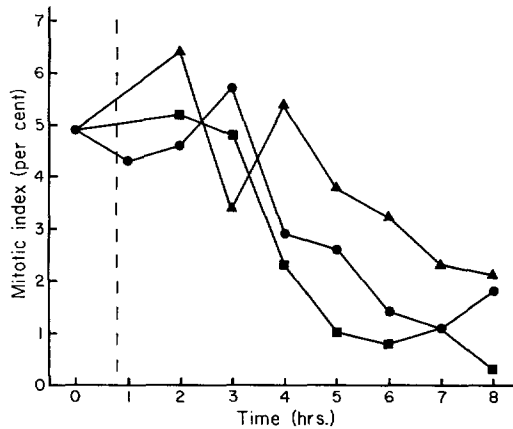


FIGURE 1

Changes in mitotic index of excised pea root tips, during exposure at 25°C to various oxygen tensions. ●, air (20 per cent oxygen); ▲, 5 per cent oxygen; ■, 100 per cent oxygen.

EXPERIMENTS ON INTACT SEEDLINGS: The seedlings were germinated for only 42 hours instead of 48, so as to obtain shorter radicles (22 to 26 mm long). One seedling was laid flat in each Warburg manometer flask, taking care not to damage the root. The flask contained 2.0 ml of water in the main well, and 0.2 ml of 2 N KOH in the center well. The required oxygen tensions were established in the flasks by the evacuation procedure (Amoore, 1961 *b*). The flasks were shaken in the manometer bath at 90 cycles/minute for 16 hours, under casual illumination. At intervals of 3 hours or more, oxygen was added through the manometer taps to some of the flasks, to replace that consumed by respiration. After the incubation the 1.7 mm root tip was excised and fixed in acetic-alcohol for determination of the mitotic index.

DEFINITIONS: The period of *physiological inter-*

phase is, for the present purposes, specially defined as ending 3 hours before the start of visible prophase. The period of *physiological mitosis* is defined as starting at the same time, 3 hours before prophase.

The *normal rates* of respiration, interphase, and mitosis are taken to be the rates occurring in air. The *relative rates* of each process under various conditions will be expressed as percentages of the normal rates.

The *rate of interphase* is the average rate of progress of cells through the period of physiological interphase. The *rate of mitosis* is the average rate of progress of cells through physiological mitosis.

Absolute time is the time between excising the root tips from the seedlings and fixing them in acetic-alcohol. *Exposure time* is the time between placing the excised tips in a changed gas or medium and fixing them.

RESULTS

Effect of 5, 20, or 100 Per Cent Oxygen on the Mitotic Index of Pea Root Tips

Excised root tips were maintained in atmospheres containing 5, 20, or 100 per cent oxygen, and samples were fixed every hour for 8 hours, for subsequent determination of mitotic index. The results are shown in Fig. 1. Zero time represents the excision of the root tips, and at 0.8 hour they were exposed to 5 or 100 per cent oxygen (as indicated by the vertical broken line). In air, the mitotic index first decreased slightly, then rose to a peak at 3 hours from excision, after which it decreased steadily for the next 4 hours, finally rising to a small peak at 8 hours. The period of steady fall from 3 to 6 hours forms the basis for the present considerations.

In 5 per cent oxygen, the mitotic index first rose at 2 hours, dropped sharply at 3 hours, and rose again equally sharply at 4 hours. Then it fell steadily during the last 4 hours of the experiment. The period of steady fall in mitotic index between 4 and 7 hours in 5 per cent oxygen corresponds with the steady fall between 3 and 6 hours in air. The delay in the fall of mitotic index represents the slowing of mitosis in 5 per cent oxygen.

When the tips were exposed to pure oxygen, the mitotic index remained near the initial value for 3 hours, then fell rapidly and steadily during only 2 hours to a low value at 5 hours, finally rising to a

small peak at 7 hours. The period of steady fall between 3 and 5 hours in 100 per cent oxygen corresponds with the fall between 3 and 6 hours in air. The advancement in the fall of mitotic index represents the speeding up of mitosis in 100 per cent oxygen.

Calculation of the Relative Rates of Mitosis in 5 Per Cent and 100 Per Cent Oxygen

Corresponding points on the final (steadily falling) sections of any pair of mitotic index curves (Fig. 1) must represent equal amounts of mitotic progress. Now, progress is directly proportional to the product of time and rate. Therefore, if the amount of progress is the same, the inverse ratio of the corresponding exposure times will give the relative rate of mitosis. For example, where the 4 per cent mitotic index ordinate intersects the curves, the exposure time in air was 2.8 hours and in 5 per cent oxygen was 4.1 hours. Hence the relative rate of mitosis in 5 per cent oxygen compared with air is 2.8/4.1, or 68 per cent. The corresponding exposure time in 100 per cent oxygen was 2.5 hours; so the rate of mitosis in 100 per cent oxygen is 2.8/2.5, or 112 per cent of the rate in air.

This calculation was repeated at $\frac{1}{2}$ per cent intervals of mitotic index, for 5 per cent oxygen compared with air, and for pure oxygen compared with air. The mean values showed that the rate of mitosis in 5 per cent oxygen was 67 per cent of the rate in air, and that the rate in 100 per cent oxygen was 122 per cent of that in air.

Calculation of the Relative Rates of Mitosis in Oxygen Tensions below 5 Per Cent

In Fig. 1, exposure time was the independent variable, with a fixed oxygen tension, *e.g.* 5 per cent. It is noticeable that a broadly similar graph of mitotic indices had been obtained previously (Amoore, 1961 *b*, Fig. 2) by taking oxygen tension as the independent variable, with a fixed exposure time, *e.g.* 4 hours. The similarity is demonstrated in Fig. 2, in which these two mitotic index curves have been redrawn, for convenience of comparison. An interesting feature of the curves is the observation that they do not descend steadily from left to right, but first fall to a low point or "trough," then rise to a high point or "peak," before descending finally towards the right. The occurrence of these features has previously been discussed in detail, and an hypothesis has been advanced to account for both high and low points (Amoore,

1961 *b*, Fig. 5). The same explanations apply to the origins of trough and peak, irrespective of whether these features are demonstrated by plotting mitotic index against oxygen tension after a fixed time, or by plotting mitotic index against time in a fixed oxygen tension. Furthermore, corresponding points on the two curves must represent equal amounts of mitotic progress.

For example, referring once more to Fig. 2, the absolute times at which the troughs appeared were noted. The appropriate (unpublished) intervals were subtracted to obtain the actual exposure times, which were 2.2 hours for the upper

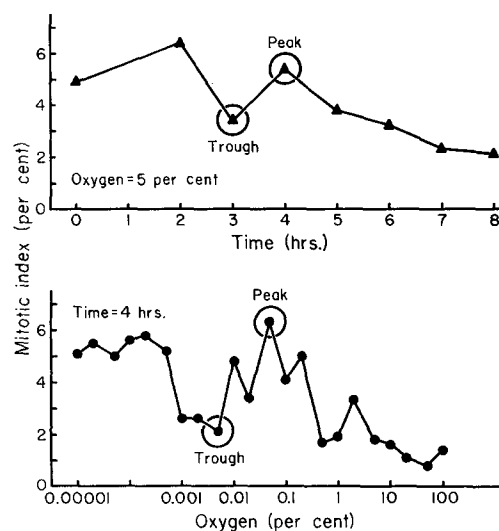


FIGURE 2

Upper curve, mitotic index of excised pea root tips incubated in 5 per cent oxygen for various times. *Lower curve*, mitotic index of tips incubated for 4 hours in various oxygen tensions. The two curves are broadly similar.

curve and 4.8 hours for the lower curve. Hence the same amount of mitotic progress had been made after 2.2 hours in 5 per cent oxygen as had been made after 4.8 hours in 0.005 per cent oxygen. The rate of mitosis in 0.005 per cent oxygen, relative to the rate in 5 per cent oxygen, is given by the inverse ratio of the exposure times (2.2/4.8) as 46 per cent. However, the rate in 5 per cent oxygen was already known to be only 67 per cent of the rate in air. Hence the product of the two rates yielded the relative rate of mitosis in 0.005 per cent oxygen as 46 per cent \times 67 per cent \times 100, or 31 per cent.

Analogous methods were used to calculate the

TABLE I

Relative Rates of Mitosis as a Function of Oxygen Tension

The last six values were obtained by recalculating data reported by Amoore (1961 *b*, Tables 1 to 4).
M.I., mitotic index.

Oxygen tension	Source of data	Experimental vessel	Independent variable	Points of comparison	Compared with oxygen	Rate relative to air
%				<i>M. I.</i>	%	%
20	Fig. 1	Sinter	Time	—	—	(100)
100	Fig. 1	Sinter	Time	Ordinates	20	122
5	Fig. 1	Thunberg	Time	Ordinates	20	67
0.35	1961 <i>b</i> , T2	Warburg	O ₂ tension	½	20	53
0.32	1961 <i>b</i> , T1	Warburg	O ₂ tension	½	20	48
0.05	1961 <i>b</i> , T3	Warburg	O ₂ tension	Peak	5	56
0.005	1961 <i>b</i> , T4	Thunberg	O ₂ tension	Trough	5	31
0.0007	1961 <i>b</i> , T4	Thunberg	O ₂ tension	½*	5	24
0	1961 <i>b</i> , T4	Thunberg	O ₂ tension	(Indirect)	—	15

* This computation, only, was based on the first fall in M.I. with increasing O₂ tension (see Fig. 2).

relative rates of mitosis in other low oxygen tensions. The results, together with some information about the source of data and the method of calculation, are collected in Table I.

Relative Rate of Mitosis in Anaerobic Conditions

It has been observed that even under the most anaerobic conditions achieved with all reasonable precautions, the mitotic index decreased appreciably during 4 hours exposure (Amoore, 1961 *b*, Table 4). Hence the mitoses were never strictly "completely arrested." The residual anaerobic rate of mitosis was calculated by an indirect method. This showed that the short phase or "block," with a relatively high oxygen requirement, must end about $\frac{3}{4}$ hour before the start of visible prophase, and not immediately before, as had been envisaged previously (Amoore, 1961 *b*, Fig. 5). From the rate of disappearance of mitoses after the above block, the anaerobic rate of mitosis was calculated to be 15 per cent of the rate in air, and this is the value entered in the last line of Table I.

Effect of Oxygen Tensions between 100 and 1 Per Cent on Mitotic Index of Pea Seedlings

The following experiment employed intact seedlings, which were incubated for 16 hours in War-

TABLE II
Effect of Oxygen Tension on the Mitotic Index of Intact Pea Seedlings

Incubation time 16 hours

Oxygen tension	Mitotic index	Relative rate of mitosis	Relative rate of interphase
(%)	(%)	(%)	(%)
20*	7.4	—	—
100	7.3	122	127
50	7.2	115	118
20	7.0	(100)	(100)
10	7.3	79	82
5	0.6	70	6
2	0.4	61	3
1	0.0	55	0

* Not incubated; initial mitotic index.

burg manometer vessels. The time of 16 hours was selected because this is the duration of the complete mitotic cycle in pea roots at 25°C (Brown, 1951). The results are shown in Table II. Between 100 and 10 per cent oxygen the mitotic index remained near 7 per cent. Nevertheless, between 10 and 5 per cent oxygen there was a very sharp fall in the mitotic index to less than one-tenth of the initial value.

Calculation of Relative Rates of Interphase in 100 to 1 Per Cent Oxygen

It was assumed that the incubation period of 16 hours was long enough for the establishment of a new steady state, as regards the distribution of cells among the several phases of the mitotic cycle. Therefore, at the end of the 16-hour experiment, the mitotic index will reflect any alteration in the relative durations of mitosis and interphase. Knowing the relative rate of mitosis at the corresponding oxygen tension, the relative rate of interphase may be calculated.

For example, the mitotic index after 16 hours incubation in 5 per cent oxygen was 0.6 per cent, compared with a mitotic index of 7.0 per cent after 16 hours in air. The ratio $0.6/7.0$, expressed as a percentage, is 8.6 per cent. However, in 5 per cent oxygen the relative rate of mitosis itself was only 70 per cent of the rate in air (this rate was read from Fig. 3). Hence, the relative rate of interphase in 5 per cent oxygen was $8.6 \text{ per cent} \times 70 \text{ per cent} \times 100$, or 6.0 per cent.

DISCUSSION

Relative Rates of Respiration, Mitosis, and Interphase in Pea Root Meristem, as Functions of Oxygen Tension

To facilitate comparison between these three functions, the relevant data were summarized in a single semi-logarithmic graph (Fig. 3). The data were assembled from Table I for rate of mitosis, from Table II for rate of interphase and from Amoore (1961 *b*, Fig. 3) for rate of respiration. Information on the relationship of all three processes to oxygen tension has not previously been available on a quantitative basis for the same tissue.

In the range 100 to 10 per cent oxygen the rates of all three processes showed a very similar proportionality to oxygen tension. However, at oxygen tensions below 10 per cent, the curve representing the relative rate of mitosis began to diverge from the other two. That is, mitosis is less sensitive to oxygen lack than is respiration or interphase. In fact, the relative rates of respiration and interphase were both restricted to 50 per cent of their normal rates by exposure to 8 per cent oxygen. In contrast, the oxygen tension had to be lowered to about 0.33 per cent in order to decrease the relative rate of mitosis to 50 per cent.

Decreasing oxygen tensions also brought about a dissociation between respiration and interphase. Whereas in 8 per cent oxygen both were proceeding at 50 per cent relative rates, in 5 per cent oxygen the rate of interphase had dropped to 6 per cent, but the rate of respiration was still 37 per cent.

Another distinction between the three processes lies in their final reactions to high degrees of anaerobiosis. Both interphase and respiration were arrested completely (slowed to less than 1 per cent of the normal rate) by anaerobic conditions, at about 1 per cent oxygen and at 0.1 per cent oxygen, respectively. However, mitosis could not be completely arrested by the strictest anaerobiosis

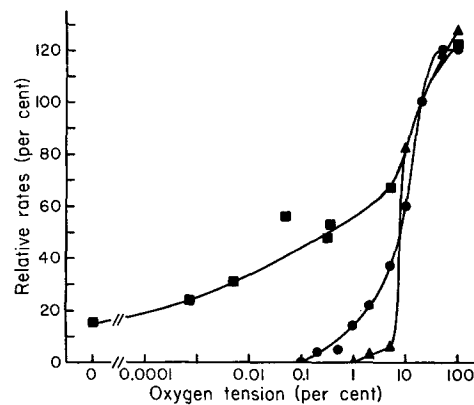


FIGURE 3

Effect of oxygen tension on the relative rates of respiration, mitosis, and interphase in pea root tips. ●, respiration; ■, mitosis; ▲, interphase.

employed, which failed to decrease the rate of mitosis below 15 per cent of normal.

The relative dependencies of mitosis and interphase upon oxygen tension were also expressed in the form of a double-logarithmic graph, analogous to those used by Hill (1910) and Wald and Allen (1957) to describe the oxygen-affinities of haem pigments. Above the half-maximum rate, both curves had a slope near 1.0. However, below the half-maximum rate, the lines representing mitosis and interphase changed their slopes abruptly, and in opposite directions. The points representing rate of mitosis lay close to a straight line of slope 0.25. In contrast, the points representing rate of interphase lay near a line of slope about 4. Therefore, in their dependence upon oxygen tension, neither mitosis nor interphase obey Hill's (1910)

equation; *i.e.* their oxygen-affinity curves are non-hyperbolic.

Thus the quantitative study of the susceptibilities of respiration, mitosis, and interphase to low oxygen tensions allows complete discrimination between them. Mitosis itself can be quite independent of respiration, as had been argued by Bullough (1952), Swann (1957), and Stern (1959). This conclusion was supported by Amoore (1961 *b*), who further pointed out that mitosis requires less than 1 per cent of the energy needed by

respiratory ferrous complex is responsible. The second is the cytological mechanism by which mitosis is arrested, but this remains obscure.

The Mitotic Cycle, and its Fluctuating Dependence upon Oxygen

The results described in this paper may be illustrated by means of a diagram of the mitotic cycle (Fig. 4). The average duration of the cycle in pea roots at 25°C is 16 hours. Visible mitosis occupies about 1½ hours, of which prophase lasts 1 hour, and metaphase and telophase ¼ hour each (Brown, 1951).

In the Methods section of this paper, the start of physiological mitosis was specially defined as occurring 3 hours before the start of visible prophase. This was done because the development of cells during that 3-hour period, like the development during mitosis itself, can proceed independently of metabolites or respiratory energy (Amoore, 1961 *b*). At this point in the cycle (3 hours before prophase) there must be a marked change in the metabolism of the dividing cell, as it moves from physiological interphase into physiological mitosis.

Wimber (1960) studied the incorporation of tritium-labeled thymidine into the nuclei of root tips of *Tradescantia paludosa* at 22°C. He calculated the durations of the mitotic (M) and synthetic (S) periods of the mitotic cycle, and also of the pre- and postsynthetic periods G₁ and G₂, respectively. If allowance is made for the rather longer cell cycle in *Tradescantia* (20 hours), the approximate durations of M, G₁, S, and G₂ may be appended to the cycle in pea roots (Fig. 4). It is apparent that the transition from physiological interphase to physiological mitosis, observed in pea roots, corresponds fairly closely with the change from synthetic to postsynthetic interphase, observed in *Tradescantia* roots.

This coincidence lends further support to the belief that the physiological demarcation between interphase and mitosis has fundamental importance. It is understandable that the full activity of respiratory processes is necessary for the synthesis of materials required for the coming mitosis. However, it is remarkable that not only visible mitosis (M), but also the whole of G₂ can progress without the continuous generation of respiratory energy.

Physiological interphase in pea roots is highly dependent upon oxygen, being slowed to half its normal rate by lowering the oxygen tension to 8

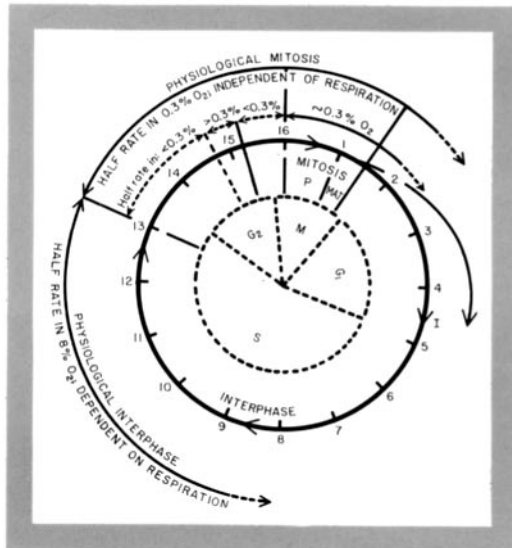


FIGURE 4

The mitotic cycle in pea roots at 25°C. P, prophase; MAT, metaphase, anaphase, and telophase; I, interphase. The postulated dependence of each phase upon oxygen tension is shown. The insertion of M, G₁, S, and G₂ is based on Wimber's (1960) results with roots of *Tradescantia*. Broken lines indicate tentative assignments.

cells to enter division. Interphase seems to be very dependent upon respiration, in this tissue with its normally aerobic metabolism. However, it appears that the susceptibility of interphase to oxygen lack depends upon some other factor besides respiration, for at lowered oxygen tensions interphase was more severely restricted than was respiration.

Special interest attaches to two important problems concerning the susceptibility of mitosis to extreme oxygen lack. The first is the chemical identity of the cellular constituent which reacts with oxygen. The following paper (Amoore, 1962) presents strong evidence that some non-

per cent. It is not known whether the whole of physiological interphase has a uniform dependence upon oxygen tension, because the method used to determine the relative rate of interphase would give the average rate. In contrast, physiological mitosis is much less dependent upon oxygen than interphase, requiring a lowering of oxygen tension to about 0.3 per cent in order to slow it to half the normal rate. The methods of determining the relative rates of mitosis should give the average rate over the whole sequence of development from G_2 to telophase. The stages from prophase to telophase were apparently uniform in their sensitivity to oxygen lack. However, the preceding G_2 should be subdivided into at least three phases (Fig. 4). Whereas visible mitosis is slowed to half the normal rate in about 0.3 per cent oxygen, most of G_2 is apparently less susceptible to oxygen lack, so that a lowering of oxygen tension to much less than 0.3 per cent would be necessary to slow it to half the usual rate. However, there is a short period, ending $\frac{3}{4}$ hour before visible mitosis, which is more susceptible than visible mitosis to oxygen lack,

that is, considerably more than 0.3 per cent oxygen is required for half the normal rate to be maintained during this sub-phase.

The experiments and calculations described in this paper show that some refinement is required of Fig. 5 in the previous paper (Amoore, 1961 *b*). Thus, 0.33 per cent oxygen, not 0.05 per cent, suffices to decrease the rate of progress of cells through visible mitosis to one-half. Furthermore, the short period of relatively high sensitivity to oxygen lack does not immediately precede prophase, but ends about $\frac{3}{4}$ hour before the start of visible prophase. However, there are not yet enough data available to attempt a complete refinement of that figure, which still serves to show, in principle, how the fluctuating dependence of the cells upon oxygen during G_2 can give rise to abnormal distributions of cells among the visible phases of mitosis.

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