THE OXYGEN CONSUMPTION OF THE MICROSPORES OF TRILLIUM IN RELATION TO THE MITOTIC CYCLE*

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Up to the present time, investigations of the oxygen consumption of dividing cells have centered either on proliferating tissues containing a comparatively high percentage of dividing cells, or on a variety of animal eggs, before and after fertilization. For several reasons, including the lack of tissues homogeneous with respect to the stage of division, the information thus obtained has been largely confined to the grosser aspects of respiratory behavior. To those changes more closely allied to the development of the mitotic cycle there exist only scanty references, although such information must certainly be fundamental. It is from this standpoint that the anthers of many plants provide excellent material for study, because, as previously indicated (Stern, 1946), the remarkably slow rate of division of the pollen mother cells and the microspores allows for a more exact determination of the changes occurring at successive stages in the development of the nuclear cycle.

Recently, Erickson (1947) made the first attempt to elucidate this type of problem by measuring the oxygen consumption of excised anthers of *Lilium* in Fenn microrespirometers. His results constitute a broad survey of the oxygen consumption of the anther during its entire development, and clearly suggest that marked changes occur at the time of division of the pollen mother cells and the microspores. Our approach, though largely similar to that of Erickson, differs somewhat in respect to the method of referring the Qo_2 values obtained. Rather than using time after planting, and length of flower bud as reference points, the oxygen uptake of the anthers was referred to the stage of division in the microspores as determined from acetocarmine smears. It is of interest that the results of this investigation confirm and supplement those of Erickson.

In order to correlate most advantageously the oxygen consumption of anthers with the stage of division of the microspores, the anthers were studied singly. Each anther was weighed, its oxygen consumption measured, and a smear preparation made to check the stage of division. This procedure not only allowed for corrections in those cases where anthers of the same bud were at different stages of development, as did occasionally occur, but it also provided

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for a check on the degree of variability within a plant. Since *Trillium* anthers at the microspore stage weighed 4 to 10 mg., the use of the microrespirometer previously described (Stern and Kirk, 1948) was of distinct advantage.

Measurements of Oxygen Uptake

The *Trillium erectum* (L) plants used in these experiments were obtained commercially and kept throughout at 3°C. This treatment appeared to have no inhibitory effect on the development of the microsporangial tissue; in fact, in nature, division takes place under a carpet of snow. Respiratory measurements, however, were made at 25°C., and in order to assure the tissues being at thermostat temperature, all plants were kept overnight at room temperature before being used. Just what the effects of this sudden change in temperature were, was not studied. It

FIG. 1. Diagram of quartz helix balance. For explanation see text.

appears nevertheless that rigid adherence to the procedure given yielded Q_{O_2} values which truly represented, from a relative standpoint, the normal behavior of the developing anther.

In every instance, the oxygen uptake of each of the six anthers of a bud was measured simultaneously in six microrespirometers. Fifteen minutes were allowed for equilibration, and readings were then made after 15 minutes and after 1 hour. In line with Erickson's observations, there was little change in the rate of oxygen consumption for the duration of the experiment. At the end of 1 hour, the anthers were weighed and acetocarmine smears were prepared to determine the stage of division.

Weighing of Fresh Anthers

In order to obtain the correct fresh weight of anthers, it was necessary to weigh the material rapidly to minimize the loss of moisture. This was performed very conveniently by use of a quartz helix balance shown in Fig. 1. The quartz fiber helix was made from uniform fiber about 0.1 mm. in diameter, wound to a total length of about 40 cm. It was suspended inside a glass tube 30 mm. in outside diameter and

with a telescoping tube at the bottom, 34 mm. in outside diameter. A small quartz cradle served to support the anther. The elongation of the spring which is, with quartz helices, exactly proportional to the weight applied, was read with a simple cathetometer. Rapid weighings to 0.01 mg. were readily possible, since the spring elongated about 6 mm. per mg. The readings were independent of temperature fluctuations within narrow limits because of the extremely low coefficient of expansion of fused quartz.

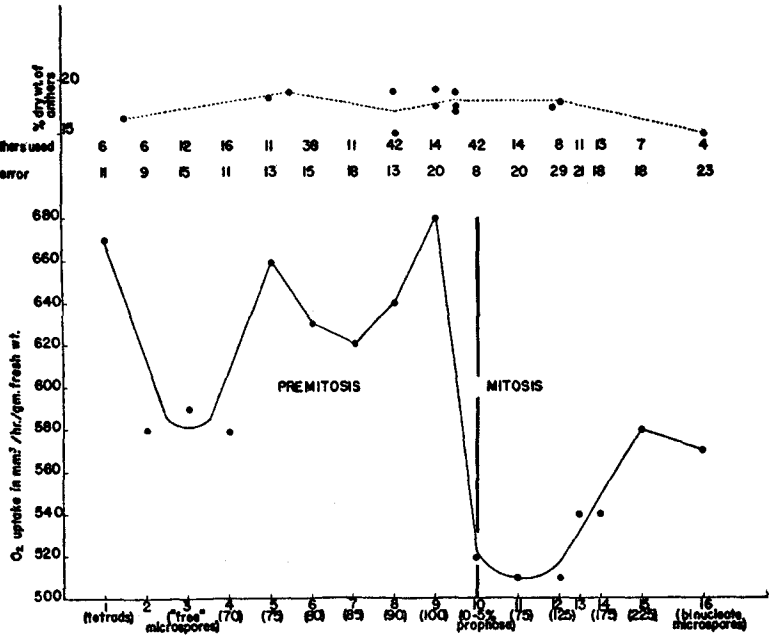

FIG. 2. The oxygen consumption of anthers during development of microspores. The bracketed numbers in the premitotic part of the scale indicate average diameter of microspores (arbitrary units); those bracketed in the mitotic part indicate the degree of development as explained in the text. The dotted line represents the changes in hydration of the anthers, each point having been obtained from studies of five anthers.

RESULTS

A total of 265 measurements of oxygen consumption of *Trillium erectum* anthers was made as described, and was plotted against the various successive stages in the morphological development of the microspores. The results are summarized in Fig. 2, in which the over-all trend appears to consist of a rise in the premitotic stages followed by a sharp drop prior to the onset of active division. It should be pointed out that the criteria by which the trend is established are not without some uncertainty. The stages in premitotic development, during which fluctuations in the curve are apparent, could not be clearly

defined. Nevertheless, allowing for a margin of error, a fairly reliable if somewhat arbitrary scale may be set up.

Following meiosis, the quartet of microspores which are closely grouped in "tetrads" begins to "loosen," and eventually the microspores appear to be no longer clustered. At this point they are comparatively small in size, but as they mature towards active division they show an over-all increase in diameter. By no means is any one anther characterized wholly by a single size of microspore, but it is possible, quickly and without much difficulty, to estimate the average. Thus, plotted against microspore size, the Q_{O_2} values showed a progressive increase with development. One lone peak in the premitotic portion of the curve stands off from the general trend, but for the present, it may be disregarded, and the premitotic trend considered as a steady rise in oxygen uptake.

The most pronounced change occurred in the anthers containing the microspores of largest diameter. Here, clearly, the rate of O_2 uptake in some anthers was high, in others low. It was further observed that those anthers characterized by a low rate usually had a small percentage of microspores in active division; if not so with all the anthers of the bud, then at least with some of them. It was therefore decided to segregate these anthers into two groups: those in which none of the anthers in the bud showed any signs of active mitosis (stage IX), and those in which at least two of the anthers in the bud contained actively dividing microspores (stage X). The result of such a classification is apparent, and from it, it may easily be inferred that at some point *preceding* active division there is a sharp drop in oxygen uptake of the anthers.

With an increase in the percentage of actively dividing microspores, there appears to be no appreciable change in the rate of oxygen uptake. This is fortunate because translation of the data in terms of individual mitotic stages is not simple. At most times during active division, there is a distribution of cells among some or all of the various stages of development. The best that can be done, therefore, is to estimate the degree of development by calculating the relative proportions of the various stages present in the anther.

To express the development of the microspores in terms of percentage of cells in active division would be unsatisfactory since that would leave out of account the proportion of cells yet to divide and those already divided. It is much better to assign a series of increasing numbers, 0, 1, 2, and 3 to premitosis, prophase, metaphase, anaphase, and binucleate stages respectively and to sum the products of these numbers times the percentage frequency of the corresponding stages in the preparation. For example:

	Premitosis	Prophase	Metaphase-anaphase	Binucleate	Total
Frequency, *per cent*	62	19	5	14	
Number × per cent	0	19	10	42	71

The use of these numbers, of course, is merely a convenience; they are not intended as a quantitative expression of development.

In general, however, the larger the proportion of cells in the more advanced categories of division, the larger the total number; and when Q_{O_2} values were plotted in this way, the behavior of the anthers, as the microspores proceeded to completion of mitosis, could be inferred.

DISCUSSION

It may be assumed that during the period of measurement, there was little progress in the development of the microspores. The rate of mitosis is slow, for even at room temperature the transition from the premitotic to the binucleated microspore occupies at least 5 to 6 days. Obviously, the change during the interval of the experiment must be very small, and the Q_{O_2} values obtained may therefore be assumed to be representative of the stage of division as determined from the acetocarmine smears.

The values given represent the oxygen uptake of the whole anther, and it may well be asked to what extent the fluctuations observed represent the situation in the developing microspores. From twelve anthers tested, it was found that the microspores plus the antheral sap constituted approximately 35 per cent of the total fresh weight of the anther. The microspores must constitute even less. It would seem then that the magnitude of the changes observed is but a fraction of those which occur in the microspore, provided, of course, the changes are not due to the remainder of the anther. From many standpoints, changes other than those occurring in the microspore do not seem likely. The morphological development of the anther, microspores apart, is not characterized by marked changes corresponding to the respiratory ones described. Erickson, in fact, suggests a gradual diminution in rate of O_2 uptake with development, a general characteristic of maturation. On the other hand, the marked correlation of the inflexions in the curve here shown with the mitotic state of the microspores points emphatically to the microspores as the factors most responsible for the kind of behavior observed.

If it be assumed, then, that the changes are largely a reflection of the behavior of the microspores, some conclusions respecting mitotic development and oxygen uptake may be drawn. Two extremes of behavior are at once apparent: a comparatively high rate of oxygen uptake occurring some time before the onset of active division, and a comparatively low one beginning before the onset of active division and continuing through it until the termination of the cycle. In view of this, the oxygen requirements of cells in mitosis would seem to be a moot question. The conclusion, for example, that the positive correlation between high O_2 uptake and high mitotic frequency indicates a high consumption of oxygen during mitosis (Beatty, 1946) may be only partly right, for the values thus obtained may largely reflect the oxygen requirements immediately preceding active division. It would, of course, be hard to infer from

the data on microspore behavior just what constitutes "normal" oxygen uptake; most likely, both extremes lie on either side of such a value.

From the standpoint of speculation, the field is inviting—and wide open. It is hardly probable that energy requirements of cells should fall during division; indeed, the reverse is more likely so that oxygen uptake cannot here be properly regarded as indicative of respiratory activity. The immediate source of energy for active mitosis may not require molecular oxygen; in fact, the possibility of such behavior being universal is suggested by a comparison of these results with those of Rapkine (1931) on sea urchin eggs, which showed that aerobic oxidation may be inhibited without interfering with the progress of nuclear division. The problem, however, requires a more complete approach, and since more elaborate studies are at present being undertaken, further discussion is deferred in anticipation of a broader range of information.

SUMMARY

The oxygen consumption of 265 single *Trillium erectum* anthers was measured before and during the mitotic cycle of the microspores using a modified differential microrespirometer.

The results show a rising oxygen consumption of the anther in the premitotic stages followed by a sharp drop immediately preceding and during active division. It is suggested from these results that active division may be associated with anaerobic behavior and that the rapid uptake of molecular oxygen commonly associated with proliferating tissues is probably characteristic of premitotic development.

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