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Oxygen Tension a Determining Factor in the Respiration of Potato Disks of Varying Thickness

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Summary. The effect of temperature on the respiration rate of potato tuber slices has been analyzed in terms of the Arrhenius equation. Freshly cut disks, irrespective of thickness, show a linear response to increasing temperature up to 30° with an activation energy (E) of approximately 12.0 Kcal. Aged disks less than 1.0 mm thick also give a linear response with E similar to that of fresh disks. With aged disks above 1.0 mm thick there is a loss of linearity above 20° and E falls to about 4.0 Kcal indicating that respiration becomes rate-limited by a diffusion process. This departure from linearity can be corrected by raising the oxygen tension or by subdivision of thick disks to give thin slices. It is concluded that the respiration of aged disks is rate-limited by oxygen deficiency and that the inverse relationship between respiration rate and disk thickness is in large part attributable to this factor.

In common with disks from most storage roots, the respiration rate of thin slices of potato tuber tissue under suitable conditions may be increased to a level 30 to 40 fold greater than that of the whole organ. This increase occurs in 2 stages (7). The first increase is of the order of 5 to 10 fold and occurs at the moment of excision. A further 3 to 5 fold increase subsequently develops as a function of time over 24 to 48 hours. The initial increase is immediate, apparently independent of any prior metabolic events, and uniformly distributed throughout the tissue slice. The secondary increase is derived, variable in rate, and non-uniformly distributed in the slice. It derives apparently from prior changes at the RNA level (3) and its rate of development is temperature-determined (9). This secondary development is most prominently manifested in the surface cells of a disk and it is the basis for the frequently observed inverse relation between disk thickness and respiration rate (8, 15).

The question as to why surface cells, as a consequence of this secondary development, should respire more rapidly than internal cells and thus give rise to the inverse relationship between rate and thickness, has received more than 1 answer. Steward, Wright and Berry (15), who first observed the relationship, suggested that oxygen availability was the controlling factor, but recently Laties (8) argued convincingly against this explanation. Essentially the argument reduces to the question whether the respiration in thick disks is rate-limited by a physical factor, i.e., the rate of O_2 diffusion to the internal cells, or by a chemical factor, e.g., regulation by some endogenous metabolic by-product. The classical method of approaching this question is to analyze the effect of temperature on respiratory rate in terms of the Arrhenius equation (6). This method of characterising a rate-limiting step as physical or chemical has not hitherto been applied to the problem of respiratory rate regulation in storage tissue slices and the results now obtained implicate O_2 tension as a limiting factor in the age-induced respiration of thick disks.

Materials and Methods

All the experiments were performed using potato (*Solanum tuberosum* L. var. Sharpe's Express) tubers freshly dug from the Institute gardens during July and August. After the potato was washed and peeled a block of tissue was sliced with a hand microtome (American Optical Co., Buffalo, N.Y.) to thicknesses between 0.5 mm and 3.0 mm. Disks of 1.0 mm diameter were punched from the slices with a cork borer. Fifteen such disks of 1.0 mm thickness weigh approximately 1 g when freshly cut. Disks were either thoroughly rinsed for 20 minutes to remove the contents of cut cells and used fresh or they were aged for 22 hours at 25° in an aerated washing device (11) incorporating a continuous throughput of tap water [mineral content approximately 18 mg/l (10)].

Tissue respiration was determined by Warburg manometry using a Braun Warburg running at maximum shaking frequency (approximately 100 oscillations per minute). A cooling coil incorporated in the circular bath permitted measurements to be made down to 5°. A volume of tissue equivalent to 15 mm of a given thickness, (from 30 0.5 mm disks to 5 3.0 mm disks) was used per vessel. Each vessel contained 2.0 ml H_2O and 0.2 ml 10 % KOH in the center well. In each experiment 4 replicates of each treatment were employed and each experiment was repeated several times.

Since only 1 Warburg apparatus was available, the effect of temperature on respiration rate had to be followed in sequential pattern. After introducing the disks into the vessels, O_2 uptake was generally measured at 5° for 1 hour. The bath temperature was then raised by 5° within 1 to 2 minutes and a 20 minute interval allowed for temperature equilibration before measuring oxygen uptake at the raised temperature for 1 hour. This procedure was repeated at temperature intervals of 5° up to 30°. The respiration rate was shown to be linear with time at each temperature.

The respiration rate of fresh disks under constant conditions increases fairly rapidly from the time of slicing and since the experimental procedure outlined above ran over 8 hours there was an appreciable increase in the respiration rate at the higher temperature, that was only partly attributable to the elevated temperature. This difficulty was overcome by using a fresh sample of tissue for each temperature measurement, i.e., tissue sliced about 30 minutes prior to being introduced into the vessels. Controls run continuously in the same vessels over an 8 hour period showed a considerable upward divergence from the periodically renewed tissue. Since the respiration rate of aged tissue reaches a plateau, it was found that it was unnecessary to renew the tissue for each temperature change with aged disks.

The effect of increased O_2 tension on the tissue respiration was determined after gassing the vessels with 100 % O_2 for 3 to 4 minutes at a gassing rate of approximately 1 liter per minute. After being aged, disks 3.0 mm thick were each sectioned into 3 disks approximately 1 mm thick using a guillotine designed for the purpose.

Results

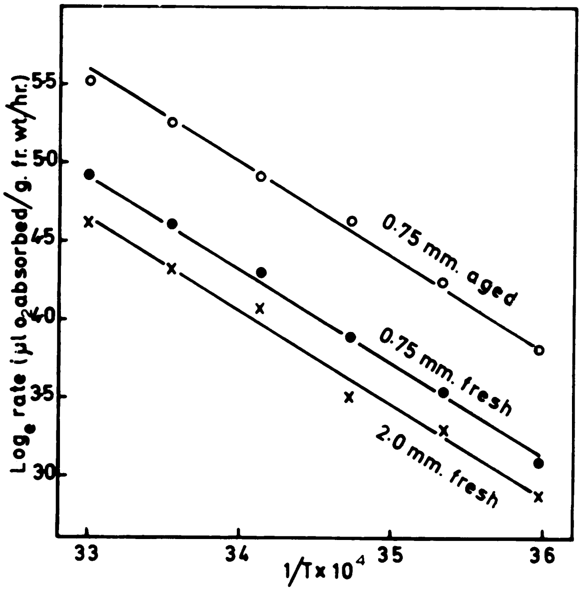

FIG. 1. The effect of temperature (from 5°–30°) on the respiration rate of 2.0 mm thick fresh potato disks and 0.75 mm thick disks both fresh and after 22 hours aging at 25°. Log$_e$K (rate) plotted against $1/T \times 10^4$. 5° = 35•97. 30° = 33•0.

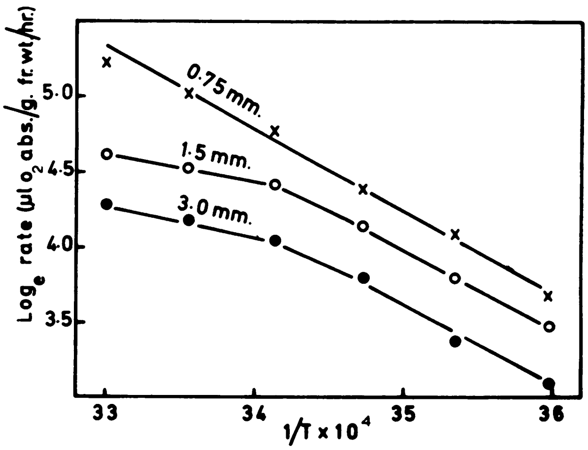

FIG. 2. The effect of temperature (from 5°–30°) on the respiration rate of potato disks of varying thickness after 22 hours aging at 25°. Log$_e$K (rate) plotted against $1/T \times 10^4$. 5° = 35•97. 30° = 33•0.

The Rate-temperature Relations of Potato Disk Respiration. The respiration rate of freshly cut disks 0.75 mm thick was measured as a function of temperature at 5° intervals from 5° to 30° and log$_e$ of the rate was plotted against the reciprocal of the absolute temperature (fig 1). When the disks were renewed with each temperature change, as explained in the Methods, a straight line was obtained. If the same disks were used throughout the temperature series the slope of the line increased slightly. When the respiration rate of aged disks (0.75 mm thick) was analyzed in the same way the points also fell on a straight line of similar slope, and this was true of fresh disks of 2.0 mm thickness (fig 1). The points plotted for fresh and aged 0.75 mm disks in figure 1 represent the average values of 4 and 5 experiments respectively. In each experiment 4 replicates were set up, each vessel containing 20 disks. The line drawn for the 2.0 mm thick disks was obtained from 1 experiment and confirmed by a duplicate experiment. Values for 0.75 mm fresh disks from the same experiment were superimposable on the 2.0 mm points.

Generally speaking fresh disks irrespective of thickness, at least up to 3.0 mm, respire at a similar rate, but with aged disks the rate per unit weight increases with decreasing thickness (fig 2). The difference is further accentuated with increasing temperature, because although thin (0.75 mm) disks show a linear increase in rate with increasing temperature, with thicker disks there is a progressive departure from linearity (fig 2).

The Effect of Increased pO_2 on the Respiration Rate of 3.0 mm Disks. A further experiment with aged 3.0 mm disks showed a loss of linearity before reaching 20° (fig 3, bottom line) but the addition of O_2 to a duplicate set of vessels (each point represents the average of 4 determinations) prevented this departure from linearity (fig 3). The same figure shows that when 3.0 mm disks are sub-divided into 3 1.0 mm segments, the outer disks so formed also show a loss of linearity which can be prevented by raising the O_2 tension. Significantly, the center disks arising from the sub-division show a linear relationship between rate and temperature, and oxygen treatment is without effect. Identical results were obtained when the temperature sequence was reversed and measurements made from 30° to 5°.

The Effect of Increased pO_2 on the Respiration Rate of Disks of Varying Thickness. Figure 4 illustrates the well-documented effect of disk thickness on respiration rate. With fresh disks the respiration rate per unit weight is virtually independent of thickness, while with aged disks the rate falls away very steeply as the disk thickness increases. The effect of increased pO_2 on disks of varying thickness is less well established. As shown in figure 4, O_2 has a slight stimulatory effect independent of disk thickness in fresh disks, but with aged disks O_2 progressively stimulates respiration as the disk thickness increases. The percent increase in O_2 uptake ranges from less than 5 % with 0.5 mm and 0.75 mm disks to over 50 % with 3.0 mm disks.

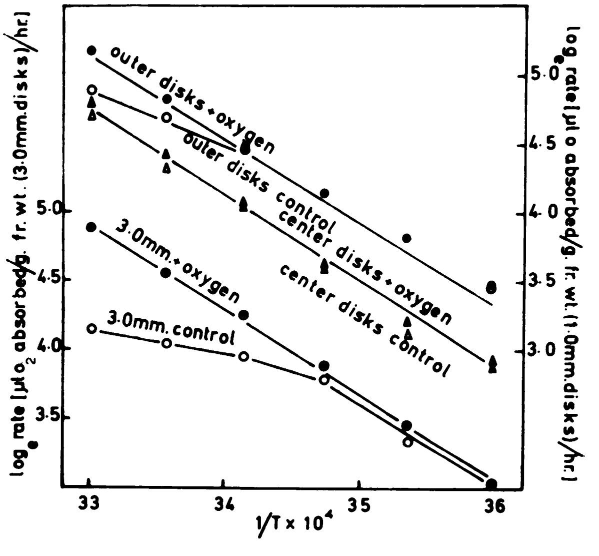

FIG. 3. The effect of increased oxygen tension on the respiration rate at different temperatures of aged 3.0 mm thick disks and on the respiration rate of outer and inner segments obtained by slicing 3.0 mm aged disks. $\text{Log}_e K$ (rate) plotted against $1/T \times 10^4$. 5° = 35•97. 30° = 33•0.

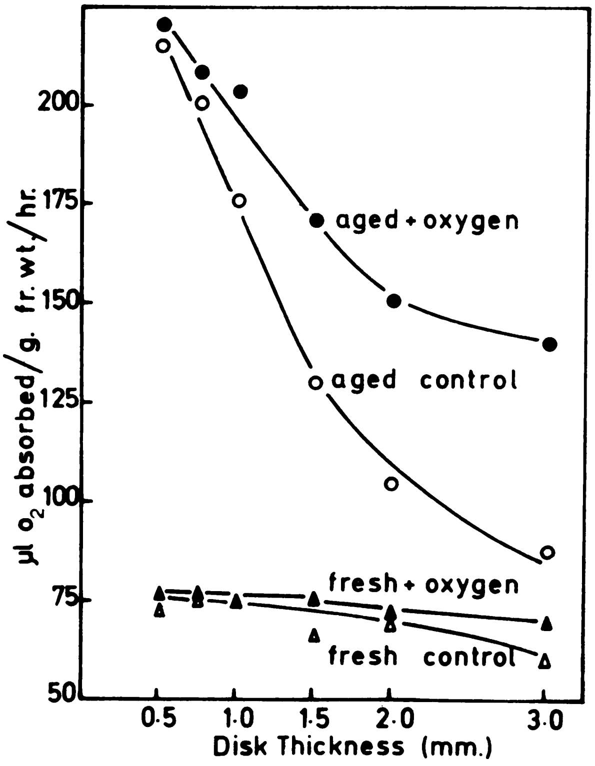

FIG. 4. The effect of increased oxygen tension on the respiration rate at 30° of fresh and aged potato disks of varying thickness.

The Effect of Further Sub-division on the Respiration Rate of Aged Thick Disks. If the respiration rate of the inner cells of thick disks following aging is restricted by oxygen deficiency as implied by these results, further sub-division of aged thick disks should immediately release the inhibition. Table I compares the respiration of whole 3.0 mm

Table I. *Oxygen Uptake as a Function of Temperature*

Aged 3.0 mm disks and outer and inner sections taken from aged 3.0 mm disks were used.

Temperature	5°	10°	15°	20°	25°	30°
	µl O_2 absorbed/g fr wt hr^{-1}					
Whole disks	20	28	45	53	58	65
Center sections	19	23	37	60	77	113
Outer sections	32	45	62	83	106	130

disks at different temperatures with that of center and outer disks obtained by sub-division of the aged 3.0 mm disks. It is clear that when these results are expressed on a unit weight basis the total respiration of the tissue is substantially raised so that at 30° it is effectively doubled. It should be noted that 2 such cuts in a 3.0 mm disk result in a loss of fresh weight of almost 20 % due to cut cells and this must be taken account of when calculating the respiration rate per unit weight.

Discussion

The relation between the rate of a reaction and temperature may be expressed in terms of the Arrhenius equation,

$$\log_e \frac{K_2}{K_1} = \frac{E}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$

in which K_1 and K_2 are velocity constants at absolute temperatures T_1 and T_2, R is the gas constant and E is the activation energy of the reaction. In simple chemical reactions E is effectively constant and a plot of $\log_e K$ against $1/T$ will give a straight line from the slope of which E can be evaluated. The constancy of E in plant respiration has been disputed (see 5). Certainly the temperature range over which it can be determined is narrow and since respiration involves a sequence of reactions, possibly with differing temperature coefficients, a lack of constancy would not seem unreasonable. Whatever significance attaches to the results shown in figure 1, and it would be presumptuous to argue from the parallelism of the lines that the respiratory pathway in fresh and aged tissue was one and the same, it is nevertheless of interest that E has the same value for both tissues and that it is effectively constant over the range 5° to 30°. By contrast, results recently reported by Coursey, Fellows and Coulson (4) for yam disks show a tremendous variation. An Arrhenius plot of the 4 hour values from their graph gives a straight line between 5° and 15° with $E = 21.2$ Kcal, and another straight line between 20° and 30° ($E = 12.8$ Kcal), while from 15° to 20° the activation energy exceeds 60 Kcal. It is probable that with these disks, O_2 uptake at the low temperatures was insufficient to give accurate readings. Results obtained from potato disks by MacDonald and Laties (12) using a sensitive oxygen electrode method, also show a constant E with a similar slope for both fresh and aged tissue from 0° to 25°.

Of more interest for our present purpose is the departure from linearity shown by thicker disks (fig 2) after aging at 25°. Thin (0.75 mm) disks give a value for E of approximately 12.0 Kcal (fig 1 and 2) which is typical of a chemical reaction, whereas for thicker disks at higher temperatures (fig 2), E is of the order of 4.4 Kcal, and corresponds to a Q_{10} of 1.27. Moelwyn-Hughes (13) states that when a diffusion process is rate-limiting the critical increment should be about 4.5 Kcal at 25°, and since the Q_{10} for diffusion rates fall within the rather narrow limits 1.2 to 1.3 (6) the obvious inference to be made is that the respiration of thick disks following aging is rate-limited by a diffusional step, and most probably by the diffusion rate of oxygen to the internal cells. On this assumption, fresh disks irrespective of thickness, yield a constant E within the chosen temperature range because the respiration rate is relatively low, but the increased respiration associated with aging is such that only in thin disks can oxygen penetrate sufficiently rapidly to permit maximum uptake. Indeed even with 0.75 mm disks, values of $\log_e K$ at 30°, consistently fell below the line (fig 1, 2).

One way of testing this assumption is to subdivide a thick disk into 3 thin disks when an increased oxygen uptake would be expected as a result of the shortening of the diffusion path. Table I confirms this expectation. Since it might be argued that the increased total respiration of segments of a thick disk represented the stimulation evoked by a wound response or by the release of a volatile inhibitor, as for example CO_2, the assumption that oxygen tension was the rate-limiting factor was further tested by raising the pO_2. This increase in the concentration gradient increased the diffusion rate of oxygen into the tissue and a linear response to temperature was obtained (fig 3). Significantly, center segments taken from whole disks give a linear increase without additional oxygen (fig 3) because their respiration rate was insufficient to restrict seriously the diffusion of oxygen, while the outer segments gave indications of oxygen deficiency due, doubtless, to their high respiratory rate.

Laties (8) examined the relationship between respiration rate and tissue thickness in potato disks and concluded that it is the development of the increased respiration and not respiration per se which is controlled by tissue thickness and that this control was effected by a negative feedback process involving a volatile respiratory product. His argument is a compelling one when his basic premise is conceded, namely, that increased oxygen tensions have no effect, or more precisely, no differential effect on aged disks of varying thickness. In his experiments the effect of oxygen at 30° was tested only on disks up to 1.5 mm thick. The present results (fig 4) show that at 30° an appreciable effect is observable at 1.0 mm (15 % stimulation), and beyond 1.5 mm it becomes substantial (over 50 % stimulation at 3.0 mm). Steward (14) reported only a 10 % stimulation, in 100 % oxygen, of the CO_2 output from aged 1.0 mm potato disks and he surmised, doubtless correctly, that in his experiments temperature (23.2°) was the rate-limiting factor. It is significant that the inverse relationship between rate and thickness only becomes apparent at higher temperatures. When the relationship between disk thickness and respiration rate shown for aged control disks at 30° in figure 4 is repeated at 5° temperature intervals down to 5° a family of curves is obtained in which, as the temperature drops, the curve becomes less exponential until at 5° a line very similar to that for fresh tissue is obtained. This seems to imply that at low temperatures the diffusion rate of oxygen to the inner cells is such as to support the maximum respiration of which they are capable at that temperature. This is also borne out by figures 2 and 3 where it is evident that oxygen diffusion does not become limiting until 15° to 20°. (The diffusion rate of oxygen is not of course significantly affected by a rise in temperature but the increased respiration resulting from a rise in temperature, makes a greater demand on the available oxygen, and the quantity diffusing to the internal cells will be reduced). From 20° upwards however the oxygen requirement by the outer cells is such that little or no oxygen (at atmospheric pressure) can diffuse past them to the inner cells where, as the temperature and thickness is increased, conditions become increasingly anaerobic. Evidence of fermentation can be found in thick chunks of tissue washed at 25°.

The obvious deduction to be made from the experimental evidence is that oxygen availability exercises a controlling influence on the respiratory pattern of potato disks. This, basically, was the view of Steward, Wright and Berry (15). They supposed the oxygen content of a whole tuber to be considerably less than atmospheric and they assumed the nature of the restraint in the whole tuber, and in the thick disk, to be one and the same. This view was challenged by Laties (7, 8) who appreciated the significance, with respect to this question, of Burton's analysis of the gas content of potato tubers. Burton (2) showed that the oxygen tension at the center of a whole tuber was not far short of atmospheric, and that in any event, the efficiency of the aerating system within a tuber is such that even a slight decrease in the partial pressure of dissolved oxygen within the tuber, will increase both the rate of intercellular diffusion and physical solution of oxygen sufficiently to meet the respiration requirement for oxygen by the tuber at least up to a temperature of 25° and probably higher. In addition he demonstrated that the respiration rate of the whole tuber was not increased at 25° in 100 % oxygen. Laties concluded that the stimulus caused by slicing was not due to an elevated oxygen tension and he postulated the existence of a volatile inhibitor to explain both the increased respiration induced by slicing and the rate/thickness relationship which develops with aging. Like Steward et. al. (15) he assumed that the restraint undoubtedly experienced by the internal cells of a thick disk is one and the same with that inherent in the uncut tuber.

Laties is certainly correct in his contention that the metabolic inactivity, relatively speaking, of a whole tuber is not to be ascribed to oxygen deficiency. Burton's analyses (2) amply justify that. Woolley (17) from a quite different experimental approach also concluded that oxygen was not a limiting factor in whole tubers or fresh tissue. By the same token neither is the immediate respiratory increase consequent upon slicing, due to improved accessibility of oxygen. Whether or not a volatile inhibitor is the effective agent in the whole tuber remains unproven. The results reported here cast no light on that problem.

It may be objected that if the rate-limiting factor is indeed oxygen tension, the rate/thickness curve in the presence of raised oxygen tension (fig 4) should not be of the same general shape as the control, i.e., an approach to an asymptote. But to suppose that with added oxygen the rate/thickness curve should fall on a horizontal plane, is to ignore the fact that the respiration changes induced by aging have not proceeded uniformly throughout the disk. The increased respiration induced by aging, involves the synthesis of respiratory capacity, and this development is most pronounced in the surface cells. It may occur uniformly in all the cells of a 0.5 mm disk but in a 1.5 mm disk there must be a considerable proportion of the cells in which the respiratory machinery has not been synthesised to anything like the same extent. It follows, therefore, that while added oxygen will allow a 1.5 mm disk to respire maximally, the maximum rate per unit weight of which it is capable is very much less than that of a 0.5 mm disk. Hence the approach to an asymptote.

These considerations suggest that when storage tissue cells are released from repression by slicing, the derepression stimulus is experienced by all the cells, irrespective of the thickness of a disk at least up to 3.0 mm. As the metabolic development associated with aging proceeds, the demand for oxygen increases to the point where, with oxygen at atmospheric pressure, the outer cells of a disk utilize all the available oxygen. With the acceleration of metabolism in the outer cells, the inner cells experience a corresponding decrease in oxygen supply, with the result that in these cells the metabolic changes characteristic of aging in thin disks, are rapidly and increasingly repressed. Consequently the addition of oxygen 24 hours later cannot elicit from thick disks the quantitative characteristics of thin disks. The transformation cannot be instantaneous. It is implied in these remarks that the threshold thickness at which thin disks begin to manifest the characteristics of thick disks, may be increased by aging disks in the presence of added oxygen, and this is currently under investigation. Meantime 2 pieces of evidence may be cited in support of the claim that the derepression signal is perceived by all the cells after slicing. In the first place, Laties (8) has pointed out that during the first 8 hours after slicing, respiration increases throughout the tissue mass irrespective of disk thickness. At the end of this period during which the respiration rate rises considerably, the inverse relationship between rate and thickness develops. Secondly, Vaughan and MacDonald (16) have shown that invertase, which is a particularly useful marker since it is not detectable in freshly cut disks of beetroot but is rapidly synthesised during aging under aseptic conditions (1), is synthesised even in the center of a 3 mm disk during the early stages of aging. But whereas this synthesis continues in the outer cells, it is greatly restricted in the inner cells.

The conclusion to be drawn from the evidence presented here is that the respiration rate of aged disks upwards of 1.0 mm thick is rate-limited by oxygen deficiency and that the inverse relationship between respiration rate and disk thickness is attributable to this factor, the influence of which is exerted both on the development of respiratory capacity and on the full utilization of the existing capacity. Earlier work has established beyond doubt that oxygen deficiency is not the effective agent in the suppression of metabolic activity in the whole tuber. Nor is oxygen rate-limiting in the respiration of fresh disks. It follows therefore that the restraint obtaining in the whole tuber is not identical with that obtaining in the internal cells of aged thick disks, nor are the factors responsible for the induction of metabolism following slicing necessarily identical with those regulating the subsequent development of that metabolism.

Acknowledgment

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