Controlling Influence of Thickness on Development & Type of Respiratory Activity in Potato Slices [1]

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Early studies of the respiratory behavior of potato tuber slices showed the specific respiration rate to be inversely related to thickness in slices up to 3.0 mm thick, and to approach a constant low value with increasing thickness thereafter [(38); see (27) for a brief summary of respiratory studies relating to slices of bulky storage organs in general]. The observed relationship was explained on the basis that a shallow superficial layer of actively respiring tissue borders tissue of uniform low activity, so that with increasing slice thickness the active layer is increasingly diluted, and the specific respiration rate thereby lowered. Steward (35, 38) postulated that the elevated respiratory activity at the surface of a disk or slice is the consequence of enhanced oxygen availability, a point of view shared with some modification by Boswell and Whiting (3) and Stiles and Dent (42). Steward further specified that where respiration rate and disk thickness are inversely related, oxygen availability determines the depth of the active surface layer rather than the level of respiratory activity throughout the tissue mass (35, 38, 41).

Several considerations call for a reappraisal of the above-mentioned classical and generally accepted view. First, the earliest experiments dealing with the relation between thickness and respiration rate showed that the respiration of potato disks in the range where rate and thickness are reciprocally related is not enhanced by increasing the external oxygen concentration above that in air (36, 41). The assumption that the active surface layer is determined by oxygen availability is thus put in question. The depth of the active surface layer was estimated by Steward (35) on the presumption that the enhanced surface respiration decreases with oxygen tension with distance from the surface, and terminates where the oxygen concentration drops to zero. In disks of 1.0 mm or less in thickness maintained in water the calculations indicated virtually the entire disk to comprise the active surface layer. However, as will be shown, the observed relationship between thickness and respiration rate [fig 1 & (38)] is inconsistent with the preceding deduction. Where the active surface layer comprises but a fraction of the disk, the foregoing view ostensibly affirms that the surface respiration is aerobic, in distinction to the subsurface, or bulk, respiration, which is anaerobic. Since respiratory rate was described solely in terms of CO_2 evolution, the term respiration was presumably used generically to include both the aerobic and anaerobic production of CO_2. However, since the respiratory quotient of potato tuber tissue is essentially 1.0 regardless of mass [see James (16)], it is evident that if oxygen tension in any way regulates respiratory activity in potato slices, control must relate to the magnitude or type of aerobic respiratory activity, rather than to the presence or absence thereof [see Choudhury (7)].

Some 70 years ago Devaux (9) recognized that oxygen, though diminished in the heart of bulky storage organs, is nevertheless plentiful, and recently Burton (5) has elegantly demonstrated that the p_{O_2} in the center of an average potato tuber is usually in excess of 0.15 atm. Although Devaux, and subsequently others, have cautioned about the water injection of air spaces in tissue slices, it will be shown below that injection is virtually not at issue in the behavior of potato disks, and thus the demonstrable prevalence of oxygen in large masses of potato tissue mitigates against the likelihood that oxygen availability controls the respiratory rate in potato slices. Since, nevertheless, potato disk respiration is clearly a function of the surface to volume ratio under certain circumstances, gas exchange may still be at issue in the determination of respiratory rate, albeit oxygen in all probability is not the gas in question. Boswell and Whiting (3) and Stiles and Dent (42) have maintained that CO_2 tension plays a part in the control of disk respiration, while Scott (32) suggested that high endogenous CO_2 levels depress the respiration of intact fleshy storage organs [cf. Richards (29)]. However, as will be demonstrated, the concentration of CO_2 in disks is no better a reason for the inverse relationship of respiration rate and thickness than is the internal oxygen concentration, and another explanation must be sought.

Thornton (45) has suggested that metabolic products other than CO_2 inhibit potato tuber respiration although, contrary to what is now indicated, such products were deemed to arise as a consequence of

[1] Received revised manuscript April 16, 1962.

[2] The work reported herein was generously supported by the National Science Foundation.

partial anaerobiosis. Steward et al. (40) noted that more than 7 % of the carbon evolved by respiring potato disks consists of something other than CO_2. Apropos of the foregoing observations the thesis is developed below, that respiratory magnitude and quality in potato disks is controlled by a negative feedback system in which the regulatory agent is a volatile metabolite other than carbon dioxide (19). A model exemplifying the hypothesis is presented in the last section.

A source of ambiguity relating to the respiratory changes which occur in storage organ slices has been the failure to distinguish the respiratory rise which occurs immediately upon cutting from the respiratory increase which develops through hours or days after slices have been prepared. In potato, for example, the respiration rate of fresh slices is *at once* approximately five times that of the intact tuber, while an additional three- to fivefold increase takes place subsequently in the course of a day. When whole tubers are peeled or quartered (29, 17, 15), bruised (28), irradiated (43), ventilated (32), or exposed to cyanide (13), they may achieve a respiration rate approximately that of fresh slices. However, the respiratory rise which attends the aging of slices is restricted to thin pieces of tissue, and is not to be confused with the augmented respiration of tubers or tuber segments obtained in any of the previously designated ways. Explicitly or otherwise it is usually the respiratory increment which develops with time which is at issue when the respiration of slices is compared with that of tissue in bulk. Whether the respiration of fresh slices is qualitatively similar to that of the intact tuber is uncertain. However, there is every indication that the respiration which develops with time in thin slices is different in kind from the major part of the initial respiration (21, 22). Since the developed respiration is an apparent prerequisite to the onset of a variety of physiological processes including protein synthesis, cell division, starch degradation, and salt accumulation (38, 40) the means whereby its development is naturally regulated are of special interest and are examined herein.

Materials & Methods

Commercially available potato tubers of the variety Russet Burbank were washed and halved. Blocks of tissue 1.5 to 2.0 inches on a side were cut from within the vascular ring. Each block was pierced six times normal to the median plane with a No. 4 cork borer, the borer being inserted two-thirds of the way through the block. Slices 1.0 mm thick were cut with a hand microtome, and the disks formed thereby (0.9 cm in diam) were freed from the slice by gently dipping the latter into ice water.

When comparing disks of different thickness, one slice of the thickest member of a given series was cut first, followed by the consecutive removal of ever thinner slices, the number in each case being inversely related to the thickness. The series was repeated as often as necessary, disks of each thickness thus arising from throughout the parent block. The disks were briefly rinsed, transferred to an 80 mm Buchner funnel, and bathed for 20 minutes with 3 liters of distilled water which passed by gravity flow through the stem of the funnel and out over the funnel walls.

An appropriate number of disks (approximately 1.0 g fr wt) were transferred to standard Warburg flasks containing 2.0 ml 10^{-4} M $CaSO_4$. At the same time disks were placed in liter erlenmeyer flasks (10 g per 100 ml 10^{-4} M $CaSO_4$) and gently shaken on a New Brunswick Gyrotory shaker. In time-course studies, respiration was followed in the Warburg apparatus for some six to eight hours after which new sets of disks were taken from the shaker. The latter were rinsed and transferred to Warburg flasks, and respiratory measurements were continued. When disks on the shaker were maintained at the same temperature as disks in the Warburg bath (30 C), their respiration rate at any time upon transfer to manometer vessels was the same as that of disks kept in respirometers from the beginning. By taking new disks from the large sample after six to eight hours, contamination was avoided, and the course of respiration could be followed for 16 hours. All measurements were in duplicate, each series being repeated two or more times.

Disks to be aged for a day before use were first washed as indicated above, and then shaken on the Gyrotory shaker with three to four changes of solution during the 24-hour period. Where incubation in the presence of CO_2 and bicarbonate was desired, the disks were maintained in a tissue washer in which solution was continuously renewed while a stream of the appropriate gas gently agitated and aerated the disks (21). Alternatively, the selected gas phase was introduced into an evacuated liter sidearm flask containing 100 ml of CO_2-equilibrated bicarbonate solution. Disks were then added to the solution, and gassing continued for several minutes.

In the text to follow the term, respiration rate, will invariably mean specific respiration rate, i.e. the rate per unit mass of tissue. Disk dimensions given in millimeters will invariably refer to disk thickness.

Results & Discussion

► I. Relationship of Thickness & Respiration Rate. Although it has been virtually axiomatic that the respiration rate of relatively thin disks of potato, as well as of other fleshy plant storage organs, is inversely related to thickness, the fact remains that the respiration of freshly prepared slices is either independent of, or but slightly affected by, thickness [fig 1 & 2, cf (28)]. On the other hand, aged disks[3] display the reciprocal relationship commonly imputed

[3] The term "aged" denotes simply the passage of time.

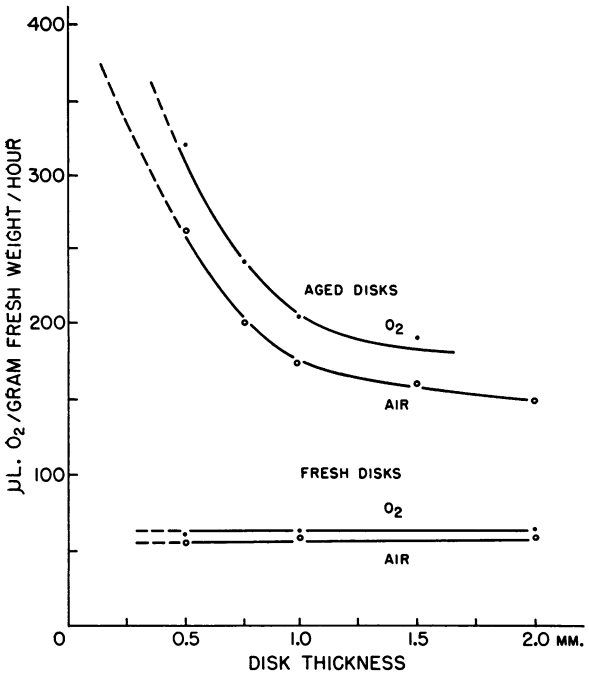

Fig. 1. The relationship of respiration rate to thickness in fresh and aged disks. Disks aged in 10^{-4} M $CaSO_4$ in air. Respiratory measurements in air or 100 % O_2 as indicated.

to disks in general [fig 1, cf (38)]. If the respiratory activity of potato slices were controlled by oxygen availability, as frequently postulated, either a decrease in the diffusion coefficient of oxygen owing to the water injection of intercellular spaces, a change in terminal oxidase characteristics involving an increase in the apparent Michaelis constant (K_s) for oxygen, or an endogenous enhancement of the respiration rate with aging could result in the establishment of an inverse relationship between rate and thickness where none existed before. In any event it is to be expected that whatever the reason for oxygen inadequacy within a tissue slice an increase in the external p_{O_2} must enhance oxygen availability, and thereby increase the thickness threshold beyond which respiration diminishes with thickness. As may be seen in figure 1, the expectation is not realized. Apart from a small respiratory increment which is independent of thickness, the same thickness-rate relationship is noted in the presence of pure oxygen as is observed in air. Thus oxygen availability cannot be responsible for the phenomenon at issue.

An attempt was made purposely to inject water into the intercellular gas spaces. To this end disks in water were successively placed under vacuum and returned to atmospheric pressure several times. As can be seen in figure 2, the respiration rate remains unaffected by this process. Actually, the intercellular gas space is of such fine dimensions that water under 5 atmospheres of pressure cannot be forced through a 5 mm potato disk when such a disk is used in place of a filter pad in a Seitz filter. The air spaces in potato apparently are in the form of Jamin's chains [see Haberlandt (12)] (alternating columns of liquid and gas within micro-conduits) where capillary forces are sufficiently great so that the water is not readily dislodged.

It cannot justifiably be argued that disks fail to show the consequences of purposeful water injection because they are fully injected during their preparation. Fresh potato disks would be anoxic at distances little more than 1.0 mm from the surface if there were no air spaces (see below). In fact there is no indication that the respiration of fresh potato tissue is limited by oxygen availability even in disks of considerable thickness. The lowest respiration rate depicted for fresh disks in figure 2 is still approximately twice the rate for intact tubers (fig 3), while the respiration rate of whole peeled tubers approaches that of 1.0 mm fresh disks. Burton (5) has neatly demonstrated that the oxygen concentration in the gas phase in the center of sizable potato tubers at room temperature is from 50 to 75 % that of air. Since the diffusion coefficient of oxygen in air is more than 10^5 times that of oxygen in water, it is evident why less than 1 % intercellular gas space in potato (5) permits adequate oxygenation of tubers, let alone tissue slices. Steward et al. (38) specifically examined the question of whether water penetration affects the respiratory behavior of potato slices. The respiration rate of relatively thick disks aged in air was found to be less than twice that of disks aged in water, rather than many times greater, as would be expected if injection were a serious problem.

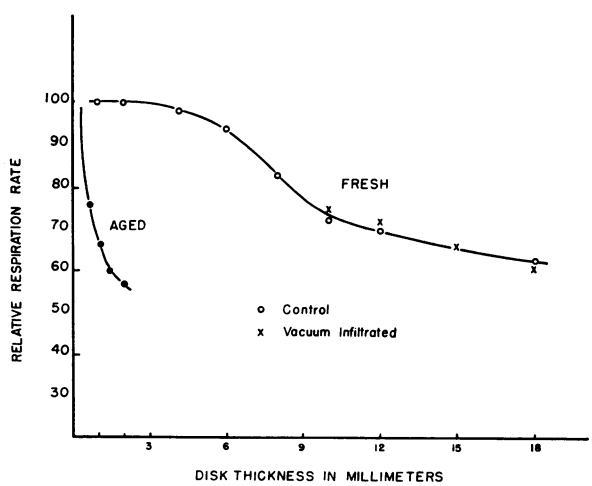

Fig. 2. The effect of thickness and infiltration on the respiration rate. Vacuum infiltration described in the text. Disks 1.2 cm in diameter. Absolute scale for aged disks approximately 4 to 5 X that for fresh. Respiration rate of 1.0 mm fresh disks approximately 35 μliter O_2/g fr wt/hr.

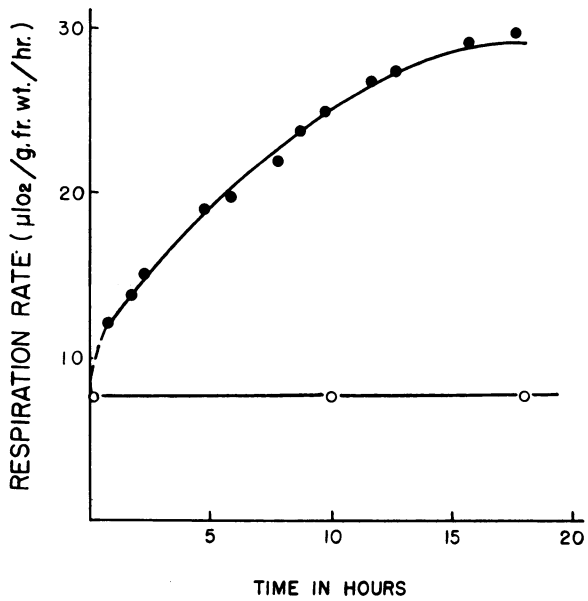

Fig. 3. The time-course of respiration in a peeled whole tuber. *Open circles,* intact tuber; *closed circles,* peeled tuber. Tuber fresh weight approximately 250 g. Two tubers per respiratory container. Respiratory measurement made with Beckman Oxygen Analyzer.

Regarding a second possible cause broached above for the development of an inverse relationship between respiration rate and thickness with aging, it is to be noted that the apparent K_s for oxygen has been shown to be virtually the same in aged and fresh tissue in spite of the fact that the sensitivity to respiratory oxidase inhibitors is markedly different in the two cases (44). Since neither pronounced water injection of air spaces nor a change in oxygen affinity attend aging in potato slices, it is pertinent to examine the expected consequences of an endogenously evoked rise in respiration rate.

When respiration is controlled by the availability of oxygen in a tissue slice, the relationship between respiration rate and thickness is expressed by a variant of Fisk's law, viz. $H = \sqrt{\frac{8D\ (C_o - C)}{\alpha}}$, where H represents thickness in centimeters, α, the respiration rate in ml per g fresh weight per minute, C_o, the external oxygen concentration in atmospheres, C, the oxygen concentration in the middle of the slice, and D, the diffusion, or invasion, coefficient (16) in ml O_2/cm^2/minute, with an oxygen gradient of 1 atm per cm. In fresh potato disks α is approximately 0.8×10^{-3}, while D, taking into account the fraction of a percent of gas space in the potato, may be as high as 1.8×10^{-2} [see (5)] rather than approximately 3.4×10^{-5}, the value for oxygen diffusion in water frequently utilized erroneously in the estimation of gas diffusion in plant tissues [see (16)]. Taking into account the ample levels of oxygen within potato tubers at ordinary temperatures, the respiration rate of a thin slice could be more than 1,000 times that of the tuber without the development of an oxygen shortage, other things being equal. Regardless of the value of D, however, the equation above defines the relationship between respiration rate and disk thickness. Thus on the basis of the unwarranted assumption that the oxygen tension in the middle of a 3 mm fresh disk, for example, is just high enough to support maximal respiration, and presuming that the respiration rate rises ninefold with aging (a maximum value obtained from figure 1 by comparing the estimated rate for an aged disk one cell, or 0.1 mm, thick, with the rate for fresh disks), it follows that so long as the thickness of an aged disk is no greater than 1/3 H initial, or 1.0 mm, respiration should be independent of thickness. Figure 1 contradicts the expectation, and theoretical considerations thus confirm the deduction that p_{O_2} does not control the respiration rate in potato slices, a deduction made on the basis of the respiratory response to elevated oxygen tensions.

While the evidence is against oxygen availability as the factor which determines respiratory magnitude in aged potato disks, the respiration rate nevertheless increases with the surface to volume ratio. However, the foregoing relationship is also consistent with the repression of respiration by a diffusible endogenous metabolite. Since with respect to the enhancement of respiration with time it matters relatively little whether slices are aged in solution or in air, the thesis is posed herein that if respiration is inhibited by an endogenous metabolite, the latter must be volatile. The statement of Fick's law set down above may be used to describe the relationship between disk thickness and the internal concentration of an endogenously produced volatile if the appropriate symbols are taken to apply to the latter, and the value, β, the rate of evolution of the volatile product, is substituted for α. Thus if it be presumed that a volatile respiratory product inhibits respiration when above some critical concentration, and if it be postulated that the concentration of said product in the middle of a 3 mm fresh slice is just at the inhibitory threshold, it follows that if the respiration rate were to rise ninefold with aging, no respiratory inhibition should be manifest in aged disks 1.0 mm or less in thickness. It follows therefore that neither the p_{CO_2}, nor the concentration of any volatile metabolite whose production is directly proportional to the total respiration, can any more be thought causative to the inverse relationship between thickness and respiration rate than can the p_{O_2}.

► II. Heterogeneity of Potato Disk Respiration. The foregoing contention regarding the regulation of respiration by a volatile metabolic product leaves open the possibility that the latter is related to but some part of the total respiration, where the respiratory metabolism is of more than one kind. In this connection both kinetic and biochemical evidence indicate

the respiratory quality of aged disks is largely different from that of fresh.

Figure 4 depicts the time course of the respiratory rise as a function of thickness. It is to be noted that the rise is sigmoidal with time rather than hyperbolic. The outstanding feature of the depicted phenomenon is that the respiration rises *throughout the tissue mass,* regardless of disk thickness, for the first 8 hours, during which time the respiration rate is approximately doubled. At this point the rise with time abates in the thickest disk of the series, while the respiration in thinner disks continues to rise sharply. As the respiration rate mounts, increasingly thinner disks successively approach a respiratory ceiling, until the thinnest disk of the series achieves its maximal rate. If the respiratory rate were inhibited by a product of the total respiration, it would be expected that when inhibition was just perceptible in the thickest disk of the series, the respiration of a disk half the thickness could rise to four times,

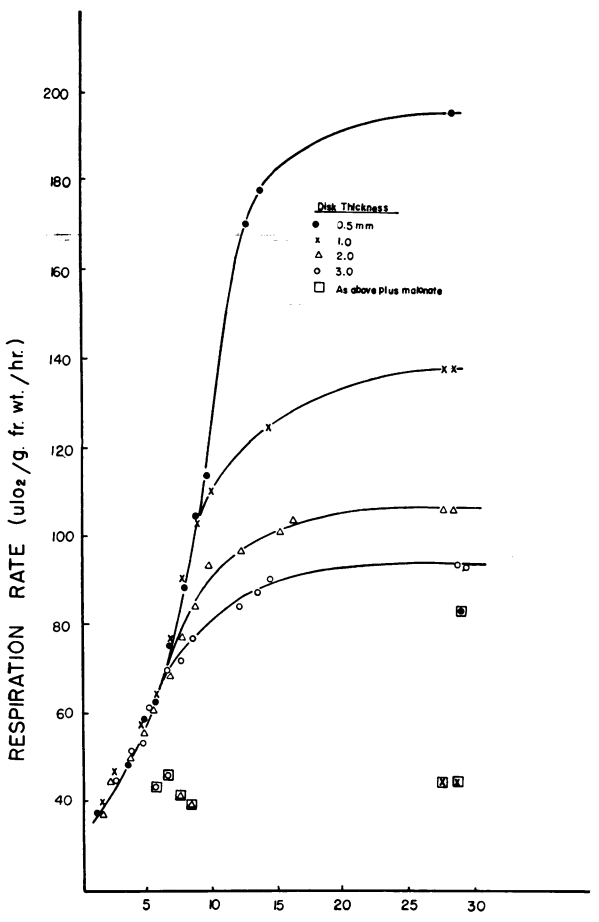

Fig. 4. The development of respiratory activity with time in disks of different thickness. Malonate added to duplicates of each control at designated time to a final concentration of 0.05 M, pH 5.0.

and of a disk one-third the thickness, to nine times that of the thickest disk, before comparable inhibition were noted. The data of figure 4 are at variance with this expectation. If, however, some part of the respiratory increment which develops with time were qualitatively distinct from the bulk of fresh tissue respiration, the distinct component could increase in magnitude many times while the total respiration increased but three- to fourfold. Under such conditions a diffusible respiratory product could control respiration at the same time that the relation between disk thickness and total respiration rate appears to contradict expectations from Fick's law.

Figure 4 and table I indicate that in distinction to the respiration of fresh potato tissue, the respiratory increment which arises with time is predominantly malonate sensitive. The developing malonate sensitivity has led to the suggestion that the respiratory increment differs qualitatively from the initial, or basal, respiration [see (20)]. However, evidence for the contention transcends this single observation and is treated elsewhere (21, 22, 24). In brief it may be pointed out that: A, Regardless of the extent to which the respiration has risen, malonate returns the respiration to approximately a common level. The minimal malonate sensitivity demonstrable in fresh potato disks is not enhanced by raising the malonate concentration. B, Fresh disks release $C^{14}O_2$ from C_6 labelled glucose poorly or not at all, while aged disks vigorously evolve $C^{14}O_2$ when presented with the same substrate. Malonate sensitivity arises concomitantly with the onset of $C^{14}O_2$ evolution from glucose-6-C^{14} (31). C, The labelling of the organic acids of the tricarboxylic acid cycle following the presentation of uniformly carbon-labelled glucose, and the effect of malonate on this labelling, is markedly different in fresh and aged tissue in a way which is consistent with the proposed hypothesis (23, 24). D, The utilization of carbon labelled glucose increases roughly 3,000 times with aging, while the respiration increases but three- to fourfold. The evolution of respiratory $C^{14}O_2$ attending the provision of glucose-U-C^{14} is almost completely repressed by malonate in aged disks, while the total respiration is inhibited less than 60 %. $C^{14}O_2$ evolution is unaffected by malonate in fresh tissue (23, 31).

The suggestion that negative feedback is involved in the course of respiratory regulation in potato slices is inherent in figure 4 and in the foregoing discussion; that is, the rise in respiration creates a condition which ultimately serves to limit the respiration rate. It is of particular interest that the limitation, which is a function of disk thickness, is imposed on the *development* of an enhanced respiratory capacity, and not on the respiratory activity per se. Thus, when cores of potato are aged, and millimeter disks subsequently cut therefrom, it is only the surface disk which suffers an appreciable respiratory rise. When, furthermore, a 2 mm disk is split in three following aging, the total respiratory activity of the three slices

Table I

Relative Malonate Sensitivity of Fresh & Aged Potato Disks

		Respiratory activity μl O_2/g fr wt/hr			Malonate inhibition	Factor of increase		Ratio relative increase
		Total	Resistant	Sensitive	%	Resistant	Sensitive	Sensitive/ resistant
Fresh	(F)	40	38	2	5			
Aged	(A)	166	106	60	36	2.8	30	10.7
	F	48	41	7	15			
	A	165	93	72	44	2.3	10	4.4
	F	48	38	10	21			
	A	115	46	69	60	1.2	6.9	5.7
	F	48	35	13	27			
	A	160	58	102	64	1.7	7.9	4.6
	F	48	41	7	15			
	A	138	45	93	67	1.1	13.2	12.2
	F	43	42	1	3			
	A	166	83	83	50	1.9	83	43.8
	F	40	32	8	20			
	A	181	72	109	60	2.2	13.7	6.2

Flask contents: $CaSO_4$, 10^{-4} M, KH_2PO_4, 10^{-2} M, K malonate 5×10^{-2} M, pH 5.0. Final volume 2.5 ml. Tissue, 1.0 g, 0.2 ml 10 % KOH in the center well. Temperature 30 C.

Table II

Effect of Disk Origin on Respiration Rate

Experiment	Condition during aging*	Thickness & origin for respiratory measurement	Respiratory rate μl O_2/g fr wt/hr
	...	1.0 mm, fresh disk	38
	1.0 mm disk	1.0 mm	170
		1.0 mm; core face	137
		1.0 mm; 2nd mm from face	48
		3rd "	43
1	Core	4th "	40
		5th "	41
		6th "	39
		1.0 mm; core face	106
		1.0 mm; 2nd mm from face	31
2	Core	3rd "	30
		4th "	32
3	2.0 mm disk	2.0 mm disk	135
	2.0 mm disk	2.0 mm disk, sliced in three	139

* Cores 1.5 cm in diameter, 3 cm. long. Following slicing, disks 0.9 cm in diameter punched from center of larger disks. All tissue other than fresh aged 24 hours.

is equivalent to that of the intact disk (table II). Since in almost all early work relating to the interrelation of disk thickness and respiration rate, disks cut to different thickness were routinely washed for a day before use [see (*27*)], it is likely that the reported effect of disk thickness on respiratory activity in fact described the effect of thickness on respiratory development. The same arguments which rule out the controlling influence of p_{O_2} on respiratory activity apply to the effect of p_{O_2} on respiratory development. Table IV contains direct experimental evidence to show that development is not enhanced by an increase in p_{O_2} during aging.

► III. Further Kinetic Considerations. It having

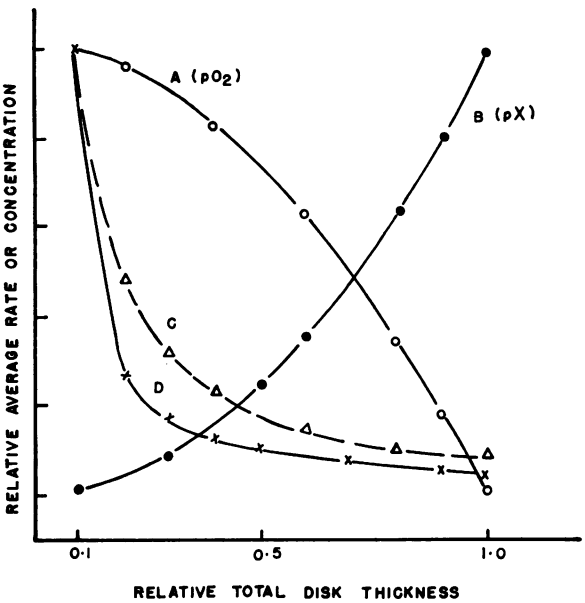

Fig. 5. Idealized relationships between disk thickness and either respiration rate or the concentration of diffusible respiratory components. The generalized equation $C = C_o + \frac{\alpha x^2}{2D} - \frac{\alpha bx}{D}$ describes the relation between C, the p_{O_2} at any distance, x, from the surface of a disk, α, the respiration rate, and b, the half-thickness $\left(\frac{H}{2}\right)$, when respiration is constant across the disk (see 14). C_o is the external p_{O_2}. When at some distance b′ (greater than x) p_{O_2} achieves a critical concentration C_i, at which respiration ceases, the expression is modified to $C = C_o + \frac{\alpha x^2}{2D} - x\sqrt{\frac{2\alpha(C_o - C_i)}{D}}$. For the first equation the average p_{O_2} is given by $C_o - \frac{\alpha b^2}{3D}$. For the second case, $p_{O_2} = C_i + \frac{1}{b}\left(\frac{C_o - C_i}{3}\right)\sqrt{\frac{2D(C_o - C_i)}{\alpha}}$.

Curve A presents the average p_{O_2}, and curve B the average p_X as a function of disk thickness where X is an endogenous metabolite produced uniformly throughout the disk (see text). Curve C depicts the expression, Relative respiration rate $= \frac{1}{b}$, where the respiration is confined to a surface layer and b varies from 1 to 10. Curve D presents the solution of the first equation above when x is solved in terms of b, where x is that distance from the surface at which p_{O_2} or p_X is equal to a given value, with all other factors kept constant. The lower limit of relative thickness is taken as finite rather than zero to indicate that in reality the thinnest disk to be considered is a disk one cell thick[4].

[4] The author is indebted to Dr. Jack Dainty for the rigorous formulation of the appropriate diffusion equations, only part of which are presented here.

been maintained that the determination of respiratory capacity, and as noted above, quality, cannot be controlled by a product of the total respiration, it must be noted that the postulated heterogeneity of potato slice respiration still is not enough to explain the relationship between thickness and respiration rate observed in figure 1. Considerations which follow lead to the conclusion that in all but the thinnest disks suppression of respiratory development must in time be almost complete at relatively short distances from the disk surface, with the result that the respiratory rise is predominantly associated with a thin surface layer.

Figure 5 depicts limited and idealized relationships between disk thickness and p_{O_2} or p_X (where X is a respiratory product formed uniformly throughout the disk) and between thickness and respiration rate, where certain restrictive assumptions are invoked. The shapes of the curves are of paramount interest. Curve A describes the manner in which p_{O_2} will vary, and curve B the way the average p_X will vary with thickness, on the assumption that the oxygen tension is greater than zero throughout, and that respiration or respiratory development is essentially independent of oxygen tension. If respiration were proportional to p_{O_2}, the p_{O_2} would drop less sharply, and p_X rise less sharply, with distance from the surface than otherwise, but the way in which the average concentrations of O_2 and of X changed with thickness would bear a generic resemblance to curves A and B, respectively [see (10)]. It follows that in those instances where the active surface layer is presumed to comprise the entire disk and the respiration to be proportional to p_{O_2} (38, 35), the relationship between respiratory rate and disk thickness should resemble curve A. In fact, the observed relationship has invariably been found to be similar to curves C or D (38).

Table III

Effect of Slicing per se on Respiratory Development During Aging

Incubation conditions*	Experiment No. 1	2	3	4
	μl O_2/g fr wt/hr			
Fresh	35	...	45	38
Aged in solution	126	116	126	126
Appressed in air	47	41	...	...
Spaced in air	111	...	...	...
Appressed in tuber	...	...	59	...
Spaced in tuber	...	...	84	71

* Disks 3.3 cm in diameter aged as described in the text; disks 0.9 cm in diameter punched therefrom after 24 hours. For slices aged in tuber, disks 1.9 cm in diameter inserted between core ends without external pressure. Core ends sealed with paraffin or held in place with rubber tape. After 24 hours, disks 0.9 cm in diameter punched from center of larger disks for respiration measurements.

If X is considered inhibitory to respiratory development in proportion to its concentration, and the relative respiratory activity be taken as 100 minus the percentage inhibition, the relationship between respiration rate and thickness of aged disks will be as in A, even though p_X rather than p_{O_2} represents the controlling parameter. If at some distance short of the middle of the disk p_{O_2} were to drop to zero or to a critical level below which respiration were totally restricted, A would be of the shape shown for part of its course, would pass through an inflection point with increasing disk thickness, and, if the disk were thick enough, would approach zero asymptotically. Similarly, B would be sigmoidal if respiratory development were totally repressed above some critical concentration of X (see legend fig 5). So long as X is produced uniformly throughout the tissue the shape of B, and indirectly of A, will be the same whether X is proportional to the total respiration or to a part thereof.

However, if the respiratory increment were limited, regardless of disk thickness, to a superficial layer, taken in figure 5 to be one-tenth the distance from the surface to the middle, curve C would describe the relationship between thickness and respiration rate. Curve D, in turn, indicates the way in which the depth of a surface layer in which p_{O_2} is above, or p_X below, a given value, varies with total disk thickness

Table IV

Environmental Control of Respiratory Characteristics

Experiment type	Aging conditions*	Gas phase during measurement	Respiratory activity
			μl O_2/g fr wt/hr
O_2 During aging	Air		160
	100 % O_2	Air	128
	Air		162
	50 % O_2	Air	154
	Air		146
	10 % CO_2, 20 % O_2 in N_2	Air	147
CO_2 During aging	5 % CO_2 in O_2		173
	As above, 10^{-3} M $KHCO_3$	Air	173
	10 % CO_2, 20 % O_2 in N_2		138
	As above, 3×10^{-2} M $KHCO_3$	Air	160
	Fresh	Air	54
	Fresh	5 % CO_2 in O_2	46
CO_2 During measurement	Air	Air	205
		4 % CO_2 in O_2	185
	Air (0.5 mm disks)	Air	270
		4 % CO_2 in O_2	270
	Fresh	Air	50
	Aged control		180
Li during aging	Aged, Li		70
	Fresh	Air	50
	Aged control		140
	Aged, Li		60
	Fresh	Air	40
	Aged control		140
	Aged, Li		60
	Fresh		49
	Aged control	Air	128
	Aged, Li		51
	Aged, Li, followed by 24 hr in $CaSO_4$		136
	Aged control, followed by 24 hr in Li		79

* All solutions 10^{-4} M $CaSO_4$. LiCl 5×10^{-2} M. Incubation period 24 hours at room temperature. 3.0 ml ethanolamine buffer together with glass beads in outer compartment of Dickens-Simer flasks where CO_2 is present during respiratory measurement (18).

when p_{O_2} is greater than zero throughout the disk. Curve D discloses why an event confined to the surface layers of a tissue nevertheless depends upon the total tissue thickness (compare 1.0 mm disk with 1.0 mm core face in Expt. 1, table II).

It is clear from figure 1 that the way in which thickness and respiratory development are related resembles C or D in figure 5, and not A. To the dictum that respiratory development is not regulated by a product of the total respiration must be added the proviso that the repression of respiratory development is not directly proportional to the concentration of any respiratory product, but rather is ultimately virtually complete in sub-surface tissue, the depth and the time at which repression takes place being a function of disk thickness. The diminishing respiration rate with increasing thickness does not reflect merely the interpolation of increasing quantities of inactive tissue between highly active superficial layers of fixed depth as heretofore suggested (38, 35). It is a cardinal point that both the depth and the respiratory capacity of the active layer are controlled by disk thickness. If it were depth alone the curves of figure 4 should diverge from each other at the same time. It is inherent in the hypothesis that thinner disks must develop higher rates of surface activity if the concentration of diffusible metabolite therein is to achieve the level necessary to suppress further development.

► IV. Influence of Factors Other Than Thickness. Historically, the phenomenon described herein has erroneously been called wound respiration, the implication being that the elevated respiration represents a traumatic response. Although the independence of the respiratory increase from the specific surface for the first 8 hours (fig 4) argues against the attribution of the respiratory rise to the cutting act, experiments were performed to further examine the point. Disks 1.0 mm thick and 3.3 cm in diameter were prepared in the usual way. However, as each disk was cut it was placed at once on top of the previously cut disk, with an intervening single lamina of well-washed dialysis tubing. A pile of 20 disks so arranged was gently fixed between the cork-faced jaws of a small C-clamp so that air spaces between disks were excluded. The pile was aged in a moist atmosphere together with a similar pile in which millimeter gaps between slices were provided by spacers. After 24 hours at room temperature disks 0.9 cm in diameter were punched from the center of the larger disks, rinsed, and their respiration measured. The data in table III indicate slicing per se does not evoke the respiratory rise which normally occurs. The consequences of slicing depend on the immediate environment. As a further example, a core may be removed from the entire length of a whole potato and the middle section of the core cut into slices. If the slices are reinserted into the potato flanked by the core ends firmly taped in place, the rise in slices so incubated is markedly repressed (table III).

Mulder (28) corroborates the fact that the respiration of fresh potato slices is indifferent to thickness. However, he imputes the inverse relationship noted in aged disks to the formation of wound periderm followed by oxygen unavailability. Since periderm formation in disks in moist air takes days rather than hours, and since disks maintained in solution show virtually no periderm formation (38) the foregoing, together with the experiments of table II, gainsay the origination of a diffusion barrier with age.

Although CO_2 has been ruled out as the factor determining the inverse relation between thickness and respiration rate, CO_2 nevertheless has an interesting effect on the respiratory metabolism (22) and upon growth (25). Specifically, when potato disks are aged in 5 or 10 % CO_2 in air, with or without bicarbonate, the respiration rises as in air (table IV) but the respiratory increment, in contrast to that in air, is almost entirely malonate resistant (table V). As shown in table IV, CO_2 has no effect on the respiration per se, neither in fresh nor in aged disks.

The effect of Li ion on respiratory development was examined since Li as well as CO_2 was shown to repress the normal respiratory changes in chicory (22). As in chicory, Li prevents the respiratory rise in potato slices which normally attends aging. Although Li, as CO_2, has little or no effect on the respiration as such, incubation in Li for 24 hours following the rise returns the respiration rate to a level close to that of fresh tissue (table IV). There thus appears a basis for the suggestion made earlier (22) that the metabolic events which give rise to the

Table V

Effect of p_{CO_2} on Malonate Sensitivity of Aged Potato Disks

Aging conditions	Respiratory rate		
	Control	Malonate*	Malonate inhibition
	μl O_2/g fr wt/hr		%
Fresh	42	43	0
Aged in air	166	76	57
5 % CO_2 in O_2	124	133	0
As above, 10^{-2} M $KHCO_3$	181	225	0
Air	160	79	51
CO_2**	153	160	0
Air	156	78	50
CO_2	132	148	0
Air	137	45	67
CO_2	119	109	8
Air	115	52	55
CO_2	113	108	4

* Malonate 5 × 10^{-2} M, pH 5.0; 10^{-2} M KH_2PO_4, pH 5.0; 10^{-4} M $CaSO_4$ in all flasks.

** 10 % CO_2, 21 % O_2 in N_2, 3 × 10^{-2} M $KHCO_3$.

respiratory increment must occur continuously for the rise to be sustained.

As noted earlier, the basic similarity between the behavior of disks aged in solution and disks aged in air has led to the hypothesis that a volatile respiratory metabolite affects respiratory development in potato slices. The involvement of an endogenously produced volatile in plant morphogenesis has been suggested by Skoog (34), and it is noteworthy that over 7 % of the volatile carbon evolved in the respiration of aged potato disks is something other than CO_2 (40). Amyl alcohol, which is produced by potatoes and which exerts a pronounced inhibition of sprouting of intact tubers (6), will repress the respiratory rise in potato slices at unnaturally high concentrations within a narrowly limited range (0.015–0.025 M). More promisingly, it has been found that acetaldehyde at relatively low concentrations completely suppresses the respiratory rise during aging, and that the inhibition is relieved when tissue is removed therefrom (24).

Discussion

Three major departures from the classical concepts of the basis for the inverse relationship between thickness and respiration rate in storage organ slices arise from the work presented herein. First, the controlling influence of thickness is not on the respiration rate per se but rather on the development of respiratory capacity. Second, the rise in respiration is not confined to a surface layer which is independent of disk thickness [cf (35)] but occurs initially throughout the tissue slice, the duration of the rise in any part of the disk depending upon the distance from the surface. Third, respiratory development under the given conditions is controlled by neither the internal oxygen nor carbon dioxide tensions. An additional point may perhaps be reemphasized: the respiratory rise which occurs with time in disks is to be distinguished from the increase over the tuber rate which is manifest at once upon slicing, and from the rise which takes place in whole organs following peeling or a variety of other treatments.

The sigmoidal time course of the respiratory rise in aging slices (fig 4) contrasts with the roughly hyperbolic rise which takes place in peeled whole tubers (fig 3). The basis for the initial autocatalytic acceleration of respiratory development, and for the subsequent deceleration, can only be conjectured, but it is suggested herein that the respective phenomena depend upon the essentially qualitative change in respiration which takes place following slicing. It has been contended by Laties (21) that the critical change attending aging in chicory slices is the onset of vigorous tricarboxylic acid cycle (TCAC) activity, and Romberger and Norton (31) have affirmed the likelihood that the same is true in potato. Laties presumed that low cycle activity is accompanied by a meager capacity for oxidative phosphorylation, and in this connection Loughman (26) has demonstrated that at low levels of inorganic phosphate, phosphorylation is feeble in fresh potato slices, but increases exponentially with aging.

It remains to indicate how the onset of TCAC activity may first bring about an autocatalytic rise, and then a deceleration, of respiratory development. The hypothesis offered is that the concentration of a volatile metabolite controls the events which take place, and although there is no justification at present for imputing regulation to a definite compound, it will greatly facilitate discussion to deal with an explicit model. Since acetaldehyde at low levels in fact prevents the respiratory changes under discussion, and since acetaldehyde can be demonstrated in potato slices, is palpably volatile, and is strategically disposed in the respiratory path, a model is offered in which acetaldehyde figures as the controlling agent. It is pertinent to the model that pyruvate may either be decarboxylated to acetaldehyde in potato (2), or oxidized and directly channeled into the TCAC. It is also to the point that glycolysis is meager in fresh, and vigorous in aged, potato slices (28). The sequence envisioned is then as follows.

When slices are prepared, acetaldehyde escapes, and the concentration drops to a level where the inhibition of the development of TCAC activity is released. As cycle activity develops, phosphorylation mounts concomitantly, the resultant increase in ATP availability exerting a positive feedback action on the glycolytic system. With the enhancement of pyruvate oxidation through the cycle, pyruvate is diverted from acetaldehyde formation, and another element of autocatalysis is introduced. At the tissue surface the continuous loss, however small, of acetaldehyde to the environment, results in the persistence of autocatalytic development. In deeper seated tissue, from which the diffusive escape of acetaldehyde is less expeditious, the increase in glycolytic activity finally leads to a level of acetaldehyde which suppresses further TCAC development. Since the concentration of an endogenously produced diffusible metabolite at any point within the tissue is a function of the total mass of tissue in which production is taking place, suppression of development will take place first in thicker disks, and the depth of the surface layer in which maximal activity can be achieved will be an inverse function of disk thickness. The implications of figure 5 lead to the conclusion that the layer of maximally elevated activity must be quite thin, and all experimental considerations (table II) indicate such to be the case. The latter point represents the only similarity between the hypothesis being presented and the prevalent concepts regarding disk thickness and respiration rate. Even so the similarity is minimal, and the basis for the conclusion in each case is markedly different. As indicated previously, the establishment of an inverse relationship between thickness and respiration rate following but a limited increase in the total respiration, indicates that the inhibitory metabolite cannot be produced in proportion to the total respiration, but must rather be re-

lated to the metabolic activity which develops from the time of cutting. It does not follow that the type of respiration which primarily develops with aging is totally absent in fresh tissues. The change in respiratory quality deemed necessary to explain the observed events can arise from a change in the relative contribution of coexisting respiration systems, or from a pronounced change in the steady-state conditions within one respiratory path. Romberger and Norton have investigated the pathways in fresh tissue respiration, and have given an indication of the extent to which the TCAC participates therein (cf. 1).

The physiological transformations which occur with time in slices are multitudinous, yet they may reasonably be considered to stem from the basic change in respiratory metabolism described herein. In this view alterations in the sugar status of storage organ slices with aging are a result rather than a cause of the respiratory increase (cf. 27, 40). A dramatic increase in salt absorbing capacity is one of the outstanding consequences of tissue slice aging (36, 26, see 20). Skelding and Rees (33) have studied the ontogeny of accumulation capacity in beet disks as a function of disk thickness. The development of pronounced accumulatory capacity follows a period of absorptive inactivity which is essentially similar in duration to the time required for the respiratory rise to reach its peak in beets (30, 42). Thickness determines the duration of the initial inactive period, while with disks of moderate thickness at least, the steady state level of salt uptake appears independent of thickness (cf. 39). The possibility arises that when the developed respiration in slices once surpasses a certain level throughout the slice, the salt accumulating capacity may become the same in slices of different thickness, the time necessary to do so being inversely related to the thickness. If the foregoing should be borne out, the implications will warrant attention in any consideration of the relationship of respiration to salt accumulation.

Both the profound physiological consequences which attend the change in quality and magnitude of respiration in tissue slices, and the realization that such changes are determined by distances no greater than several cell diameters, suggest that the spatial relation of cells to each other and to the environment may be an important factor in morphogenesis (see 11, 37). Although little has been said of the effect of slice thickness on the metabolism of mammalian tissues, at least one example is at hand where the metabolism at the surface of a slice has been shown to differ from that in the middle (8).

Nothing has been said of the influences which suppress the respiration of intact tubers compared with that of fresh slices, except to indicate that peeling brings the tuber rate to that of the fresh disk. The respiratory increment in a peeled tuber is certainly different from that which is evoked during aging in slices, and is presumably akin to the increment which is manifest at once in fresh disks. Respiratory control in the whole tuber may thus involve one or more factors in addition to those which determine respiratory development in tissue slices.

Summary

The respiration of potato slices kept in air increases four- to fivefold in the course of a day, and changes qualitatively as well. It has been demonstrated that it is the *development* of the respiratory increment which is controlled by tissue thickness, and not respiratory activity per se. The classical explanations for the inverse relationship between thickness and respiration rate, namely oxygen availability and wounding, have been ruled out, and the hypothesis is presented that regulation of respiratory development represents a negative feedback process in which control is effected by a volatile respiratory product.

It is argued that the product in question cannot be formed in proportion to the total respiration, and hence is not CO_2. It is suggested that the respiration which develops with aging is in large measure qualitatively distinct from the basal respiration, and gives rise to a respiratory product which in turn eventually represses further development.

Although in all but the thinnest disks the rise in respiration is most pronounced at the disk surface, it is a salient feature of respiratory ontogeny in slices of moderate thickness that the respiratory rise proceeds for some time throughout the tissue mass. As aging progresses, development is repressed in deeper seated tissue, the rise continuing in superficial cell layers and persisting longest at the surface. Both the depth of the poorly defined zone of markedly elevated activity, and the magnitude of respiration therein, are considered to vary inversely with disk thickness.

It is suggested, in view of the profound physiological changes attending the respiratory transformations described herein, and in recognition of the fact that the latter are governed by distances of but several cell diameters, that the proposed mechanism whereby respiratory ontogeny is controlled may have general implications with respect to development.

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