Respiratory Metabolism of Aerated Potato Disks

Research in several laboratories has clearly demonstrated that aerated potato disks develop an increased capacity for uptake of oxygen[1–7]. This additional respiratory capacity is not increased by 2,4-dinitrophenol[7] and is relatively insensitive to inhibition by cyanide[6] and carbon monoxide[5,6]. However, cyanide[8], low temperature (0–5° C.)[7] or absence of oxygen at normal temperature[8] will prevent the development of the increased respiratory capacity.

In view of these observations, it became of interest to us to learn whether the development of increased respiratory capacity requires the synthesis of new protein and whether the increased oxygen uptake is coupled to phosphorylation to give an increased work capacity. In our experiments (Table 1) the presence of the protein synthesis inhibitor, chloramphenicol, during the 24-hr. aeration of the potato disks prevented increase in respiratory capacity. Because the presence of chloramphenicol during the measurement of uptake of oxygen has little effect on the respiration of either fresh or aerated disks, it can be concluded that protein synthesis is a necessary part of the development of an increased capacity for uptake of oxygen.

Table 1. The Effect of Chloramphenicol on the Development of an Increased Respiratory Capacity by Aerated Potato Disks. Oxygen uptake (μl. O_2/500 mgm./hr.)

Fresh control	Fresh + chloramphenicol	Inhibition (per cent)	Aerated control	Aerated in the presence of chloramphenicol*	Inhibition (per cent)
24	19	25	105	33	69
24	25	−4	50	16	68
32	29	9	101	55	46
Av. 27	24	10	85	35	61

* Potato disks were aerated in the presence of 1 mgm./ml. chloramphenicol for 24 hr.
Each Warburg flask contained: 0·01 *M* phosphate, *p*H 6·9, 500 mgm. potato disks (1 cm. × 1 mm.), 3×10^{-4} *M* hydrogen cyanide or 1 mgm./ml. chloramphenicol, 20 per cent potassium hydroxide in the centre well. Incubation temperature, 30° C.

The phosphorylative capacity of the potato disks was measured by determining the extent of incorporation of phosphorus-32 into organic phosphate esters during the measurement of uptake of oxygen. Results obtained in this way indicate that the increment of respiratory capacity is coupled to phosphorylation (Table 2). The incorporation of phosphorus-32 into the aerated disks is not as sensitive to inhibition by cyanide as that of fresh disks. However, the phosphorylation of aerated disks is inhibited by the same levels of dinitrophenol required to inhibit the phosphorylation of fresh disks.

Table 2. Effect of Cyanide on the Phosphorylation Capacity of Fresh and Aerated Potato Disks
Incorporation of phosphorus-32 into organic esters. (Per cent of total counts in the cells)

Fresh control	Fresh + HCN	Inhibition (per cent)	Aerated control	Aerated +HCN	Inhibition (per cent)
32·6	13·7	58	23·6	30·1	−27·5
12·6	4·2	67	27·6	24·5	11·2
23·9	8·1	66	32·7	25·6	21·7
—	—	—	23·5	18·3	22·1
—	—	—	19·9	25·0	−25·6
Av. 23·0	8·7	64	25·5	24·7	0·4

Incubation with phosphorus-32 was conducted in a Warburg flask during measurements of oxygen uptake. The flasks contained 0·01 *M* phosphate buffer, 500 mgm. (fresh weight potato disks), 3×10^{-4} hydrogen cyanide and 20 per cent potassium hydroxide in the centre well. Incubation was at 30° C. for 3 hr.

These results lead us to the conclusion that the increased respiration of aerated potato disks is mediated by enzymes which are synthesized during the aeration period. Further, it appears that this additional electron transport to oxygen is coupled to phosphorylation because the phosphorylation observed in a fixed incubation time is not sensitive to a level of cyanide sufficient to inhibit 64 per cent of the phosphorylation of fresh disks. If the additional respiration were not coupled to phosphorylation, then the phosphorylation after 24-hr. aeration should still be sensitive to cyanide inhibition.

The results reported here can be accounted for by the hypothesis that cytochrome oxidase is a limiting factor in the respiration of fresh potato disks; and that on aeration, there is a net synthesis of an excess of cytochrome oxidase so that this cyanide-sensitive step is no longer limiting.

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[1] Boehm, J., *Bot. Z.*, **45**, 671 (1887).
[2] Richards, H. M., *Ann. Bot.*, **10**, 531 (1896).
[3] Lutman, B. F., *Bull. Torrey Bot. Club*, **53**, 429 (1926).
[4] Steward, F. C., *Protoplasma*, **15**, 497 (1931).
[5] Schade, A., and Levy, H., *Arch. Biochem.*, **20**, 211 (1949).
[6] Thiman, K., Yocum, C. S., and Hackett, D. P., *Arch. Biochem. Biophys.*, **53**, 249 (1954).
[7] Sharpensteen, Helen, Ph.D. Dissertation, 1953, Univ. of Michigan.
[8] Hackett, D. P., *Proc. Ann. Soc. Plant Physiol.*, **31**, xl (1956).

Ion Permeability of the Plasmalemma of the Plant Cell

Walker[1] has suggested that for *Nitella* there is evidence for a layer on the outside of the cytoplasm, a plasmalemma, which offers considerable resistance to the passage of ions. In support of this suggestion he advances two pieces of evidence—the bulk of the high d.c. resistance across the cytoplasmic layer from vacuole to outside solution of chloride lies in the cytoplasm and not in the tonoplast, and secondly, that the equilibrium concentration of calcium ions in the cytoplasm calculated from the potential difference is absurdly high.

If the tonoplast is practically impermeable to cations, then the conductivity of the cytoplasm and tonoplast will depend on the concentration and mobility of anions in these phases. If they are similar for both phases, then the bulk of the resistance will reside in the cytoplasm because of its much greater thickness. The average resistance of 6,000 ohm sq. cm. with a thickness of 10μ would be appropriate for a concentration of chlorine ion in the cytoplasm of 2·6 μequiv./l. This is the equilibrium concentration if the external solution has a concentration of 1·5 m.equiv./l. and a potential difference relative to the cytoplasm of 160 mV. If the external cation were univalent then the concentration of non-diffusible anion would be 0·87 equiv./l. The external concentration and that of non-diffusible anion would be smaller if the potential difference were less. The concentrations used ranged from 0·05 to 1·0 m.equiv., but the values for the potential difference with these solutions are not given.