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Terminal Oxidase of Orchard Grass

Abstract. The response of infiltrated surviving green leaves to HCN, 1-phenyl-2-thiourea, and sodium diethyldithiocarbamate is consistent with a functional role for cytochrome oxidase in respiration. Polyphenol oxidase does not function as a terminal oxidase in orchard grass.

The identity of the functioning terminal oxidase in green leaves of grasses has not been clearly established. While studying losses of dry matter in the curing of hay, I therefore examined the terminal oxidase responsible for respiration in the leaves of green grass. Cytochrome oxidase has been reported in various plant tissues, but the pathway of electron transfer varies among different plants (1). Polyphenol oxidase has been regarded by many as a possible terminal oxidase, although there is little direct evidence of its primary function in undamaged cells (2). However, reports that it is at least partially functional continue to appear (3), so that it appears desirable to offer contrary evidence.

Daly *et al.* (4) reported that respiration of young leaves of barley was medi-

ated by cytochrome oxidase. However, in older leaves respiration was not inhibited by carbon monoxide; thus the functional oxidase in mature leaves was left unknown. Deijs and his co-workers (5) showed that, as rye grass dried, its decline in respiration was paralleled by a similar decline in polyphenol oxidase activity. These workers attributed HCN inhibition of grass respiration to the effect of cyanide on polyphenol oxidase.

Orchard grass (var. Potomac) contains cytochrome oxidase and abundant amounts of polyphenol oxidase. Neither the intact leaf nor homogenates of it oxidizes ascorbic acid. Cytochrome oxidase appears to be the principal functioning terminal oxidase.

Grass of height 10 to 20 cm was homogenized for 40 seconds in a Waring blender with cold 0.1M phosphate (5 ml of buffer per gram of grass). For the assay of polyphenol oxidase the phosphate buffer was pH 6.5, for cytochrome oxidase, pH 6.8. Since addition of 0.2M sucrose and 0.001M ethylenediaminetetraacetate did not increase cytochrome oxidase activity, these compounds were usually omitted (6). For ascorbate oxidation, the homogenizing medium was 0.1M citrate-phosphate, pH 5.7 (7). Glass-distilled water was used throughout.

Cytochrome oxidase was manometrically measured in darkness at 30°C with 0.014M *p*-phenylenediamine as substrate. This enzyme is stimulated two- to threefold by the addition of 10⁻⁵M exogenous cytochrome *c*. The most active fraction of cytochrome oxidase is sedimented in 20 minutes at 6230 g (average). For the assay of polyphenol oxidase, oxidation of catechol was followed manometrically (8). Polyphenol oxidase activity is not sedimented by 16,700 g in 20 minutes.

Several enzyme inhibitors were studied both in vitro and in surviving leaves. Grass was cut into pieces 1 to 2 cm long and vacuum infiltrated with inhibitors in 0.1M phosphate, plus 0.2M sucrose at pH 6.8, prior to respiration measurements. Table 1 shows that the effects of inhibitors in vivo are similar to those obtained with the cytochrome oxidase preparation but that they differ from those obtained with polyphenol oxidase. The stimulation of cytochrome oxidase and leaf respiration by diethyldithiocarbamate could be caused by its acting as a substrate for this oxidase (9). The response of intact green leaves to inhibitors is consistent with a functional role for cytochrome oxidase in respiration. Polyphenol oxidase cannot be the terminal oxidase in this issue.

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Transmission of Rabies to Laboratory Animals by Bite of a Naturally Infected Bat

Abstract. An insectivorous bat that attacked a man in western Montana was induced to bite suckling mice. Subsequently the bat died, and brain and salivary gland suspensions were inoculated into other mice. Rabies virus was isolated from all three groups of mice.

In a review of the relationship between bats and rabies, Enright (1) noted that transmission of rabies by bite of insectivorous bats had not been demonstrated. Burns (2) failed to obtain transmission to monkeys, guinea pigs, and white mice by bites of experimentally infected *Tadarida mexicana* and *Antrozous pallidus*, although he found the saliva infectious by intracerebral inoculation. Stamm *et al.* (3) also found the saliva of one experimentally infected bat (*Myotis lucifugus*) infectious on intracerebral inoculation. However, what evidence exists for transmission of infection by the bites of insectivorous bats rests upon the occurrence of infection in human beings. At the present time, one well substantiated (4) and two possible (5) infections have been reported in North America. The present report records infection in white mice resulting from the bite of a naturally infected little brown bat.

The bat (*Myotis californicus californicus*) (6) was captured by elk hunters on 21 September 1958 in the Bitterroot Mountains of western Montana, where it attacked one of them twice. The first attack occurred at midday of a sunny day while the hunter was standing in camp. The bat suddenly appeared, lit on the front of his shirt, and bit the fabric. The hunter, a technician in the Rocky Mountain Laboratory, was aware

Table 1. Effect of inhibitors on oxidases and on respiration of orchard grass.

Inhibitor	Percentage change in activity from control*		
	Polyphenol oxidase (in vitro)	Cytochrome oxidase (in vitro)	Infiltrated surviving leaves
10 ⁻² M HCN	- 90	- 100	- 40
1-phenyl-2-thiourea, saturated (8)	- 100	0	0
10 ⁻² M Na diethyldithiocarbamate	- 100	+ 16	+ 60

* The endogenous respiration of grass infiltrated with 0.1M phosphate, pH 6.8, averaged 270 μ l of O₂ per hour per gram of fresh grass. Oxygen uptake per Warburg flask averaged 111 μ l/hr for cytochrome oxidase, and 330 μ l/hr for polyphenol oxidase.