

of the intact glands, are able to respond to testosterone administration in such a way that the final volume is identical in both cases. This indicates that the diminution of the nuclear volume after ligation of the excretory duct is not the result of cellular degeneration, since the nuclei respond to the same extent to pharmacological stimulation. It may be admitted that this diminution is related to the lowering of secretory activity.

In conclusion, the present study (10) reveals that an increase in the size of the nuclei of the submaxillary gland occurs after stimulation of glandular activity induced by testosterone propionate administration. This has been observed in controls and in glands in which the excretory duct was ligated. Ligation of the excretory duct significantly reduces the nuclear volume. This diminution is not related to cellular degeneration and is attributed to the lowering of secretory activity by the interruption of the salivary flow.

References and Notes

1. A. Lacassagne, *Compt. rend. soc. biol.* **133**, 227 (1940).
2. V. Valeri, *Compt. rend. acad. sci. (Paris)* **238**, 1613 (1954).
3. A. Benninghof, *Anatomische Nachrichten* **1**, 50 (1950), cited by J. Clavert, *Arch. Anat. microsc.* **41**, 209 (1952).
4. L. C. U. Junqueira, *Exptl. Cell Research* **2**, 328 (1951).
5. M. Rabinovitch, doctoral thesis, Universidade de São Paulo (1951).
6. J. F. Fernandes and L. C. U. Junqueira, *Exptl. Cell Research* **5**, 329 (1953).
7. O. Olivo, E. Porta, and L. Barberis, *Arch. ital. anat.* **30**, 34 (1932).
8. F. Yates, *J. Agr. Sci.* **23**, 108 (1933).
9. G. W. Snedecor, *Statistical methods* (Iowa State College Press, Ames, ed. 4, 1946).
10. I wish to thank Harold Deutsch for the English translation and Lucien Lison for the assistance throughout the course of this investigation.

6 July 1954.

Exudate of the Mushroom, *Polyporus dryadeus*

Benjamin Libet

Department of Physiology, University of California
Medical School, San Francisco and Berkeley

Clear reddish-brown droplets of fluid appear on the surface of a large, flat basidiomycete, *Polyporus dryadeus* (1), although this exudate is not present under all conditions in the plant's life. The exudate attracted my interest (i) because of the copious rate of production of the clear fluid (tens of milliliters were readily collected by pipetting, and this was replaced by freshly formed exudate in a matter of hours), and (ii) because shallow but definite pits were present on the surface of the plant, where the pools of exudate had been sitting, which indicated that the fluid might have a fungicidal or fungistatic action.

The exudate is slightly acid, with a pH around 5. The material responsible for the reddish-brown color is almost completely precipitated by adding 5 vol of 95 percent ethanol to the original exudate; hardly any precipitation by alcohol takes place if the exudate is

made slightly alkaline or more acid. The alcohol precipitate is not dissolved by petroleum ether or by carbon tetrachloride, but it does dissolve in ethyl ether. There is no precipitation upon addition of trichloroacetic acid to the exudate. Boiling the original fluid, with or without the addition of strong NaOH, does not alter the color. Addition of 0.1 percent FeCl₃ solution to the almost colorless supernatant fluid (obtained after alcohol precipitation of the coloring matter) gives a strong yellow color test, suggesting the presence of organic acids (2). The color of the exudate changes with alterations of its pH, becoming a light yellow below about pH 4.5 and becoming a dark brown at about pH 7 and higher.

Pigmented substances have been found and identified in some related organisms. For example, "polyporic acid" (3,6-dihydroxy-2,5-diphenyl-4,4-benzoquinone) has been extracted from *P. nidulans* and *P. rutilans* (3). But, unlike "polyporic acid," the coloring matter in the exudate of *P. dryadeus* does not give a violet color with dilute ammonia, nor is it precipitated to any extent by HCl. It also appears to have distinctly different solubility properties from the colored substances in another preparation, "polyporin," which has been described by Bose (4) as a filtrate of a culture of *Polystictus sanguineus*. It seems improbable that the pigment in the exudate of *P. dryadeus* belongs to the group of "humins" that may result from autolytic tissue breakdown of proteins containing cyclic amino acids (5). The presence of the colored droplets on the surface of *P. dryadeus* seemed to precede rather than to follow the appearance of the pits underneath them. In contrast to the pigment under study, the humins are reported to become darker and to be precipitated in HCl and also to be precipitated by alcohol from an alkaline medium.

The collected exudate did not lose its clarity or undergo any obvious changes in color for months, even though it was collected and stored (at about 5°C) under nonsterile conditions and without the addition of any antibacterial agent. Although there have been reports of a lack of antibiotic activity by *P. dryadeus* against some bacteria (*Staphylococcus aureus* and *Escherichia coli*) (6), there was no mention of whether the exudate discussed in this paper was used in the test or was being produced by the plant at the time; and evidently no test of antifungal activity was attempted. Antifungal activity has been reported for another polypore, *P. biformis* (7).

Further purification of the pigment in the exudate of *P. dryadeus* and more adequate testing of it for antibiotic activity, especially for antifungal activity, would appear to be very desirable. Of additional interest is the question of the biological significance of the pitting or apparent degeneration of the mushroom's surface in response to its own exudate.

References and Notes

1. I am indebted to L. Bonar and Ralph Emerson of the University of California, Berkeley, for identifying the organism. I also wish to thank E. Jorgensen of the College of Pharmacy, University of California, San Francisco, for consultation on some points in this paper.

2. S. P. Mulliken, *A Method for the Identification of Pure Organic Compounds* (Wiley, New York, 1904), vol. I, p. 78.
3. F. Mayer and A. H. Cook, *Chemistry of Natural Coloring Matters* (Reinhold, New York, 1943), p. 97.
4. S. R. Bose, *Nature* **153**, 292 (1946); S. R. Bose, A. B. Bose, and K. L. Dey, *Science* **107**, 63 (1948).
5. J. W. Foster, *Chemical Activities of the Fungi* (Academic Press, New York, 1949), p. 270; H. Bortels, *Biochem. Z.* **182**, 301 (1927); G. Behr, *Arch. Mikrobiol.* **1**, 418 (1930); H. Katznelson, *Soil Sci.* **49**, 83 (1930).
6. W. J. Robbins *et al.*, *Bull. Torrey Botan. Club* **72**, 165 (1945); A. H. Hervey, *ibid.* **74**, 426 (1947); L. Karel and E. S. Roach, *Dictionary of Antibiosis* (Columbia Univ. Press, New York, 1951).
7. W. J. Robbins, F. Kavanaugh, and A. Hervey, *Proc. Natl. Acad. Sci. (U.S.)* **33**, 176 (1947).

6 August 1954.

Cation-Induced Respiration in Barley Roots

Emanuel Epstein

U.S. Department of Agriculture, Beltsville, Maryland

In the accumulation of salt against a diffusion gradient, the need for metabolic energy expenditure is patent, and some linkage must exist between the accumulation of salt and the energy-yielding process of respiration. In this connection, the observation has frequently been made that when inorganic salt is added to distilled water in which plant root tissue is respiring, there is an increase in the rate of O_2 uptake and CO_2 release. Lundegårdh (1) and Robertson *et al.* (2) have shown conclusively that the respiratory system involved is the cytochrome system. However, Lundegårdh's (1, 3) theoretical model linking the increased respiration with the absorption of anions only ("anion respiration") has been questioned (4). Recent investigations in this laboratory (5, 6) into the kinetics of ion absorption by excised barley roots have led to the conclusion that the absorption of both cations and anions involves intermediate compound formation between the ions and "binding compounds" or "carriers." These findings did not support the view that the mechanisms of cation and anion absorption differ in kind, in the manner assumed in the theory of anion respiration.

Synthetic ion exchangers bearing exchangeable mineral ions offer an opportunity of exposing plant roots to absorbable ions in the absence of absorbable ions of opposite sign. Overstreet and Jenny (7) and others have shown that mineral cations and anions adsorbed in exchangeable form are readily taken up by plant roots. This paper reports experiments (8) in which excised barley roots were exposed to cations adsorbed on synthetic weak-acid and strong-acid cation exchangers, and their effect on the rate of O_2 uptake by the tissue was measured.

The weak-acid exchanger was XE-97 (9). The acid form of the resin was titrated with KOH to give a suspension pH, at the final concentration used, of 8.0. The strong-acid exchanger used was the -400 mesh form of Dowex 50 (10). The K^+ form of the resin was prepared by treating it with excess KCl, followed

by leaching with water until it was free from Cl^- . At the final concentration used, the suspension pH of K^+ -Dowex 50 was 5.0.

Barley was germinated and grown in dilute Ca^{++} solutions, as described previously (5), with minor modifications. The barley variety used in the present experiments was Atlas 46. The uptake of O_2 by the excised roots was measured by means of conventional Warburg technique; 0.40 g (fresh weight) of roots was used in a final volume of 4.0 ml distilled water or suspension. The temperature was $24^\circ C$, and the shaking rate was 165 cy/min over an arc of 2 or 3 cm. The rate of oxygen diffusion was not limiting under these conditions, and the suspensions were vigorously agitated. All treatments were set up in triplicate or quadruplicate.

Figure 1 presents the results of a typical experiment with K^+ -XE-97. Experiments with salts of monovalent ions, such as KCl or RbBr, give increases in the rate of O_2 uptake of approximately the same magnitude. The responses are similar in magnitude and time course to those obtained by Milthorpe and Robertson (11) for barley roots exposed to KCl. Exchanger saturated with H^+ ions (suspension pH 4.5) had no effect on the rate of O_2 uptake by the roots.

In the afore-described experiment, the suspension pH was 8.0, owing to hydrolysis of the cation on the weak-acid exchanger. A high pH, however, is not a prerequisite for the cation effect to be apparent. When roots were exposed to the K^+ form of the strong-acid cation exchanger, Dowex 50, suspension pH 5.0, similar effects were obtained, the only difference being that higher concentrations of K^+ -Dowex 50 were required for a given response. At a K^+ -Dowex 50 concentration of 200 milliequivalent per liter with respect to K^+ , the magnitude of the respiratory response was the same as that obtained with K^+ -XE-97 at 50 meq/lit.

The pH of the medium, however, is not without effect. When the weak-acid exchanger, XE-97, was titrated with Ca^{++} to a suspension pH of 8.0, a respiratory response was obtained which was essentially equal to that resulting from K^+ -XE-97 at the

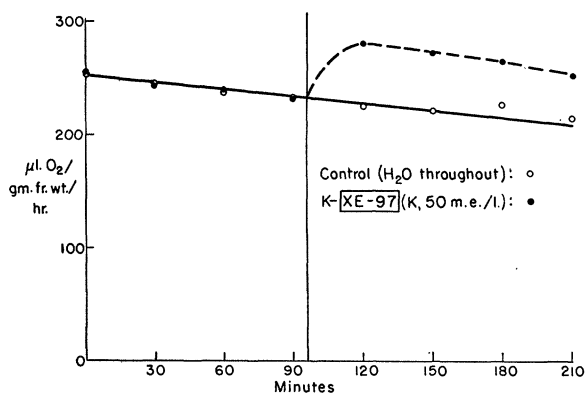


Fig. 1. Rate of O_2 uptake by excised barley roots: effect of tipping in a suspension of K^+ -XE-97.