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Cation-Induced Respiration in Barley Roots

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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 *p*H, 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 *p*H 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°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 *p*H 4.5) had no effect on the rate of O_2 uptake by the roots.

In the afore-described experiment, the suspension *p*H was 8.0, owing to hydrolysis of the cation on the weak-acid exchanger. A high *p*H, 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 *p*H 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 *p*H of the medium, however, is not without effect. When the weak-acid exchanger, XE-97, was titrated with Ca^{++} to a suspension *p*H of 8.0, a respiratory response was obtained which was essentially equal to that resulting from K^+-XE-97 at the

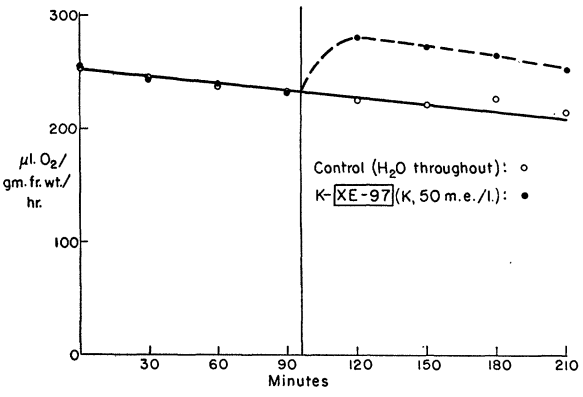

Fig. 1. Rate of O_2 uptake by excised barley roots: effect of tipping in a suspension of K^+-XE-97.

same pH. But Ca^{++}-Dowex 50, suspension pH 5.0, 200 meq/lit with respect to Ca^{++}, had no effect on the rate of O_2 uptake. The failure of Ca^{++} on the strong-acid exchanger, Dowex 50, to produce the effect is due, in part, to the smaller exchangeability of Ca^{++}, as compared with K^+, and is related, second, to the fact that Ca^{++} even as $CaCl_2$ gives rise to only a small and transient respiratory response. The results suggest that the cation-responsive system has a higher affinity for alkali cations than for the alkaline earths, and that the latter can be effective only upon removal of H^+-ion competition.

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Carbomycin, a Growth-Maintaining Factor for *Endameba histolytica* Cultures

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In vitro studies indicated that Magnamycin (carbomycin) had inhibitory effect on cultures of *Endameba histolytica* (*1*), and clinical trials showed that this antibiotic had a specific therapeutic effect in human amebiasis of the colon (*2*).

The observations reported in this paper concern the maintenance of *E. histolytica* cultures by subculturing once a week in the presence of subinhibitory concentrations of carbomycin instead of the usual or routine method of culturing every 48 hr or three times a week.

During the course of the studies on the acquired sensitivity and resistance of *E. histolytica* to antibiotics, it was observed that the ameba cultures could be serially maintained in the presence of subinhibitory concentrations of antibiotics (*3*). In the present studies, freshly prepared solutions of carbomycin were routinely used. Intravenous carbomycin hydrochloride was first dissolved in sterile saline and then serially diluted in 5 ml buffered saline overlay of N I H modification of Boeck and Drbohlov egg medium in 200-, 100-, 50-, and 25-μg/ml concentrations. Columbia strain of *E. histolytica* was selected for these initial experiments because it was a sturdy strain and had a tendency to develop resistance to antibiotics (*3*). It was serially grown in these four concentrations every 48 hr or three times a week. At the 24th subculture or generation, weekly transfers or subcultures were also started in 200-, 100-, 50-, and 25-μg/ml concentrations. At the time of the writing of the manuscript, the 48-hourly subcultures are in their 138th and the weekly subcultures are in their 40th generation or subculture.

In the 48-hourly transfers, the growth was very rich in all four concentrations, but about the 15th generation, it gradually became poor in 200 μg/ml, and about the 20th generation in 100 μg/ml concentration, nevertheless there was growth in the 138th generation. Although the growth was quite scanty, yet the amebas looked normal and were slightly undersized and sluggish. The growth in 50- and 25-μg/ml concentrations was in excellent condition in the 138th generation. A comparison of the ameba count in these concentrations with that of the stock Columbia strain showed a slight enhancement of growth, the trophozoites appeared healthier, were more actively motile, and had a tendency to be slightly larger.

The weekly transfers in 200-, 100-, 50-, and 25-μg/ml concentrations of carbomycin hydrochloride grew adequately until the 15th generation. The growth in 200 μg/ml became progressively poor from the 17th to 22nd generations and finally stopped. Similarly, the growth in 100-μg/ml concentration became quite poor at about the 26th generation and finally stopped in the 28th generation. In 50-μg/ml concentration the growth was quite satisfactory, and in 25-μg/ml concentration the growth was excellent, and there was a tendency for the trophozoites to be somewhat larger than the parent strain. An ameba count showed that the intensity of growth was almost the same as in the stock Columbia strain. At the 22nd generation, weekly subcultures were also started from 25-μg/ml weekly transfer culture, in 12.5- and 6.25-μg/ml concentrations. Presently, these subcultures are in their 14th generation. Although large numbers of trophozoites are still present, the growth is progressively getting poor.

Microscopic, macroscopic, and cultural studies of the bacterial flora of the weekly and thrice-weekly cultures with carbomycin showed no apparent variation from that of stock Columbia strain.

During the course of these experiments, subcultures from the experimental weekly and thrice-weekly cultures were serially grown in normal medium containing no antibiotic. Subcultures grew very well and did not differ from the parent stock Columbia strain, indicating that there was no carbomycin dependability.

Other strains of *E. histolytica* and other antibiotics are under investigation and will be reported at a later date.

In recent years, streptomycin-dependent strains of bacteria have been reported for *Mycobacterium tuberculosis, Neisseria meningitidis, Staphylococcus aureus, Proteus morganii, Pseudomonas aeruginosa, Klebsiella pneumoniae, Brucella melitensis, Bacillus subtilis,*