same $p$H. But $Ca^{++}$-Dowex 50, suspension $p$H 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,*

*Escherichia coli,* and *Salmonella typhi* (*4*). Apparently *E. histolytica* does not become carbomycin dependent, although in lower concentrations it enhances growth. There is no suppression of the accompanying bacterial flora in these cultures. Probably in very small quantities carbomycin appears to act as a growth-promoting or vitaminlike factor to *E. histolytica* cultures, or in subinhibitory concentrations, it inhibits or neutralizes some enzyme system or systems that induce autolysis of amebas.


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## Benzotriazole, a Plant-Growth Regulator


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Benzotriazole causes distinctive morphological modifications in the tomato plant. The changes are characterized by breaking of apical dominance, reductions in the number of leaflets per leaf, elimination of leaf serrations, cupping of the leaflets, and extensive elongation of the petioles which are unable to support the weight of their own leaves (Fig. 1). The morphological changes are limited to the leaves that developed after the initiation of treatment. Denny (*1*) has previously reported that benzotriazole hastens the sprouting of dormant potatoes.

A formative effect was produced in tomatoes only when bezotriazole was applied to the roots. The Bonny Best variety of tomatoes (*Lycopersicon esculentum* Mill.) were grown in sand culture, supplemented with inorganic nutrient. When the plants were at the 6-leaf stage 50 ml of 50 ppm benzotriazole was applied daily for 10 consecutive days to the sand surrounding the roots. Within 2 to 3 wk after the final application of benzotriazole, formative effects developed. In contrast, concentrations as high as 1000 ppm benzotriazole applied to the foliage were without effect on tomato.

The similarity in structure between benzotriazole and indole or the purine bases suggests that benzotriazole may be a competitive antagonist of either of these organic nuclei vital in biological systems. Galston and Hand (*2*) have, in fact, demonstrated that adenine enhances the response of etiolated pea epicotyls to indoleacetic acid (IAA) and that benzimidazole, a

Fig. 1. (Top) Plants treated with benzotriazole (left), untreated (right). (Bottom) Stem of treated plant (left), untreated (right).

metabolic antagonist of adenine (*3*), partially inhibits the response to both IAA or adenine (*4*).

Attempts to reverse the effects of benzotriazole with daily applications of 50 ml of either 0.5 to 50 ppm of IAA or 250 ppm adenine sulfate were unsuccessful. IAA was applied in conjunction with benzotriazole, whereas adenine sulfate applications were started 10 days prior to, and continued until 8 days after, benzotriazole applications had ceased. Although adenine did not reverse the action of benzotriazole, other purine bases or some of their ribosides would be worthy of further investigation as benzotriazole antagonists.

A number of other unsubstituted 5-membered heterocycles, both free and condensed with benzene, were applied to the roots of tomato. With the exception of benzothiazole (*5*), neither indole, benzimidazole, benzoxazole, benzothiophene, benzothiadiazole, thiophene, pyrolle, thiazole, imidazole, nor 1,2,4-triazole caused a formative effect on tomato.


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