GROWTH MODIFIERS

Antibiotics and Their Effects on Plant Growth

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These experiments were undertaken to confirm the antibiotic stimulation of plant growth under rigidly controlled conditions, to compare several antibiotics on the basis of their ability to stimulate growth, and to study the mechanism of this stimulatory action. *Lemna minor*, one of the duckweeds, was used as the test object because it can be rendered aseptic and grown under aseptic conditions easily. Its uniform growth, relatively rapid rate of growth, ability to grow on a chemically defined medium, and small size make it an ideal experimental plant. Not all antibiotics stimulate plant growth. Penicillin G and Bacitracin were outstanding in their ability to stimulate growth under test conditions. In general, most other antibiotics stimulated or inhibited growth according to the concentration tested, the length of the growth period, or the specific conditions of the experiment. The results of this investigation have suggested several hypotheses as to the mechanism of stimulatory action. It is hoped that experiments currently in progress will determine which of these hypotheses is correct.

Representatives of most groups of biologically active compounds have been tested at one time or another to determine their effects on the growth of higher plants, and especially their roles in plant metabolism. The results also furnish valuable comparisons with studies of microorganisms and animals. For example, the sulfa drugs were found to inhibit seed germination, but this inhibition could be overcome by the addition of *p*-aminobenzoic acid (*33*), indicating the similarity to results obtained with microorganisms.

Since the beginning of the "antibiotic era," the effects of antibiotics on plant growth have been tested, in general with inhibitory results. The reason, perhaps, for this type of result is that few workers tested levels below 50 p.p.m., and that these earlier preparations were impure and in many instances plant hormones more toxic than most antibiotics (*46*) were present (*4, 11, 37*). This should be kept in mind when evaluating early work with antibiotics in field of plant growth.

In addition to the well-known stimulatory effects of antibiotics on the growth of higher animals, stimulations have been reported for yeasts, bacteria, fungi (*13, 19, 20, 23*), protozoa (*6, 10*), and higher plants (*14, 18, 27, 29*). As the mechanism of stimulatory action of antibiotics is unknown, there is no way of correlating the effect of antibiotics in stimulating the growth of animals with their effect on plants.

The cytological effects of various antibiotics were studied by Wilson and coworkers (*17, 43, 44*). They found that penicillin G and endomycin had little effect on mitosis; that neomycin and circulin were without much effect on mitosis but were highly, and almost immediately, toxic; and that streptomycin, Actidione, streptothricin, Aureomycin (chlortetracycline), Chloromycetin, and Terramycin (oxytetracycline) induced mitotic aberrations of the type commonly referred to as C-mitosis. It was stressed that, because of the closeness of the mutagenic and lethal thresholds, there is little probability of proliferation of genetically changed cells.

Another effect of antibiotics on plants is the bleaching action of streptomycin resulting from the destruction of the chloroplasts. Since the report by von Euler and his colleagues (*7*), various investigators have confirmed this phenomenon for many plants (*15, 31, 32*). Schopfer and his coworkers in Switzerland have reported that streptomycin blocks the biosynthesis of carotenoids by pea seedlings grown aseptically (*34, 35*).

Several workers have shown that various antibiotics can be absorbed by the roots and stems of plants and translocated to the leaves. Plants used in such studies included soybean, lima bean, oats, lettuce, beans, and cress (*1, 5, 8, 9, 25, 45*).

The work in this laboratory on the effects of antibiotics on plant growth started with in vitro work with sorrel virus tumor tissue. After this tissue had been shown to require vitamin B_1 for growth, it was found that the addition of certain antibiotics (penicillin G, oxytetracycline, streptomycin, thiolutin, and Bacitracin) to the basal medium at concentrations of 1 to 5 p.p.m. in the absence of vitamin B_1 allowed apparently normal growth of the tissue (*27, 29*). However, this effect does not persist for more than a few transfers. Thus it seems unlikely that the antibiotic in this case replaced the vitamin or eliminated the requirement for the vitamin. Nétien and his group showed that dihydrostreptomycin at 10 p.p.m., in a medium without indoleacetic acid, stimulated the growth of carrot tissue in vitro (*26*), but this stimulatory effect did not take place in the presence of plant hormones.

Such in vitro results led to the study of the effects of antibiotics on germinating seeds under standard laboratory conditions and in sterilized soil under greenhouse conditions. Both increased germination percentages and apparent growth rates were noted for several antibiotics applied to different seeds including century plant, sweet corn, sorrel, radish, and hybrid cucumbers (*27*). There are, of course, many hurdles to clear before results from controlled experiments can be correlated with data from the field. Besides such obvious variables as climatic conditions and species variation, one must take into account the influence of soil type and content on the properties of antibiotics. Clays adsorb many antibiotics with a high degree of irreversibility (*24, 36*); the difficulties so arising are obvious (*27*).

The value of a duckweed as a test plant

for many kinds of nutritional and other physiological studies has been stressed (*38*, *40*), and its possible usefulness, particularly for antibiotic studies, has been reiterated by Hutner (*22*).

One objective in this laboratory has been to attack the problem of the mode of stimulatory action of antibiotics. In this study a duckweed (*Lemna minor*) was used as one of the test subjects (Figure 1). The duckweeds comprise a family containing the smallest flowering plants, easily rendered aseptic and easily grown under aseptic conditions. The uniform growth of *L. minor*, its relatively rapid growth, and its ability to grow on a chemically defined liquid medium, plus its small size, make it an ideal experimental plant. This presentation discusses some findings along this line, using primarily the data from the duckweed experiments.

The basal liquid synthetic medium used throughout these studies consists simply of a few salts, sucrose, three vitamins, and trace elements (Table I) (*28*). The pH of this medium is about 5.0 without adjusting. Any change in pH due to the addition of an antibiotic was corrected by the addition of sodium hydroxide or hydrochloric acid. Test solutions of antibiotics, made up at ten times the highest level to be tested, were sterilized by sintered-glass filtration, and added to the sterile basal medium aseptically to give the desired concentrations. All experiments were run in quintuplicate. Experimental transfers for each experiment were accomplished by placing a microorganism-free rosette of four fronds in each flask; all transfers for a given experiment came from one stock flask. Growth during the test period was in an air-conditioned culture room in the light with controlled temperature at 22.5° C. The results are compared here on the basis of wet weights. In general, effects on wet weight are similar to the effects on frond multiplication. As in some instances there are also effects on leaf size, it was decided to express the results in terms of wet weight.

Results of Experiments

A group of antibiotics was screened under the conditions stated, and test compounds were added to bring the concentrations to 1, 5, 10, and 20 p.p.m. The results of such an experiment after 3 weeks' growth, presented in Table II, point out the great variation in the effect of different antibiotics on the growth of the test plant. All five compounds which stimulated the growth of the virus tumor tissue also stimulate the growth of duckweed under the test conditions. Included in the screening of these more important antibiotics was the antitubercular compound isonicotinic acid hydrazide; it caused a stimulation of growth. Bustinza and coworkers in Spain, who have long been interested in antitubercular compounds, recently reported on their studies of the phytotoxicity of isonicotinic acid hydrazide (*12*). At higher levels they found only toxicity using laboratory germination tests with several plants including lupine, *Lupinus albus*, but when they tested concentrations as low as 1 p.p.m., they observed slight stimulation.

Further experimentation with oxytetracycline indicated that when the growth periods were increased the resultant stimulation was also considerably increased. This suggested that the stimulation might be due to breakdown products of oxytetracycline rather than to the antimicrobial molecule itself.

In order to test this hypothesis, 5 p.p.m. of oxytetracycline were added to a different set of flasks daily for 11 days. Three days later (or 14 days after the start of the experiment) all these sets of flasks, containing oxytetracycline of different ages with a control set containing only the basal medium, were inoculated with a rosette of four *Lemna* fronds. The duckweed in all sets were allowed to grow for 24 days before wet weights were determined. The results shown in Figure 2 demonstrate that the length of time which oxytetracycline had been in solution is a factor affecting growth.

If such an effect is due to a degradation compound, the question arises as to whether the presence of living cells would affect the rate of breakdown. In order to answer this question, three sets of flasks were set up: (A) basal medium only for control, (B) basal medium plus 5 p.p.m. of oxytetracycline, and (C) basal medium only to which oxytetracycline was later added. At the same time a stock solution of 50 p.p.m. of oxytetracycline in the basal medium was made and sterilized by filtration. This was to be added to set C 18 days after inoculation. *Lemna* rosettes were added to all three sets and allowed to grow for 18 days, at which time oxytetracycline from the stock solution was added to set C to give a level of 5 p.p.m. The plants were allowed to grow for 3 weeks longer, and then wet weights were determined. The oxytetracycline was the same age in sets B and C, but in the third set, it was not in the presence of *Lemna* for the first 18 days. The results presented in Table III show that there is no difference in sets B and C. Both more than doubled the growth of the control and one must conclude that, in this instance, the presence of living plant tissue is not a prime

Table II. Comparative Effects of Antibiotics on Growth of *Lemna minor* Under Aseptic Conditions After 3 Weeks

Compound	% Change from Control on Wet Weight Basis			
	1 p.p.m.	5 p.p.m.	10 p.p.m.	20 p.p.m.
Actidione	−85	−88	− 88	− 90
Aureothricin	−85	−95	− 95	− 95
Bacitracin	0[a]	+80[b]	+100[b]	+130[b]
Catenulin	−85	−98	− 98	− 98
Chloromycetin	+40	+10	− 45	− 75
Chlortetracycline	−10	−80	− 85	− 90
Citrinin	−10	−60	− 75	− 85
Griseofulvin	−10	−15	− 20	...
Isonicotinic hydrazide	+50[b]	+70[b]	+130[b]	+145[b]
Neomycin	−20	−75	− 80	− 90
Netropsin	−50	−55	− 65	− 75
Oxytetracycline	+10	+35	− 20	− 55
Patulin	−10	−80	− 85	− 90
Penicillin G	+40[c]	+80[b]	+150[b]	+330[b]
Polymyxin	−15	−15	− 80	− 95
Rimocidin	−10	−40	− 45	− 50
Streptomycin	+50[b]	+10[a]	− 10[a]	− 10[a]
Streptothricin	− 5[a]	− 5[a]	− 20[c]	− 20[a]
Subtilin	−25	−30	− 35	− 40
Thiolutin	+15[a]	−85	− 90	− 90

Statistical analyses not carried out unless marked as follows:

[a] Not significant at 5% level.
[b] Significant at 1% level.
[c] Significant at 5% level.

Table I. Composition of Basal Medium

KNO_3	0.002*M*	Thiamine	100γ/l.
$Ca(NO_3)_2$	0.003*M*	Pyridoxin	800γ/l.
KH_2PO_4	0.001*M*	Nicotinamide	800γ/l.
$MgSO_4$	0.001*M*	B	0.1 p.p.m.
$CaCl_2$	0.003*M*	Mn	0.1 p.p.m.
KCl	0.002*M*	Zn	0.3 p.p.m.
$MgCl_2$	0.001*M*	Cu	0.1 p.p.m.
Sucrose	2%	Mo	0.1 p.p.m.
		Fe	0.5 p.p.m.

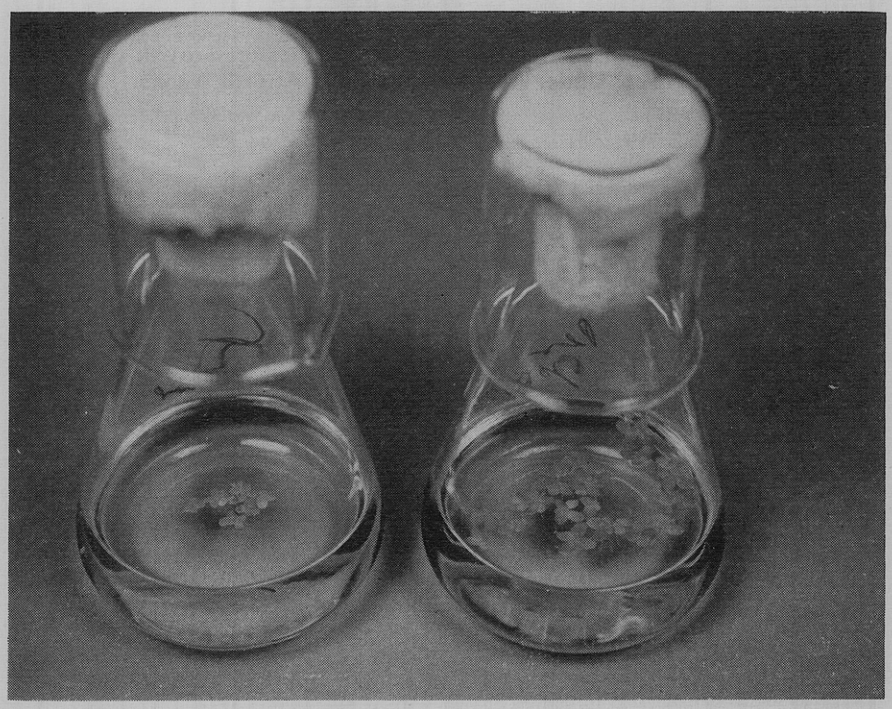

Figure 1. Duckweed (*Lemna minor*) plants growing under aseptic conditions in 125-ml. Erlenmeyer flasks

3 weeks after start of experiment. Flask at left is control (basal synthetic medium only). Flask at right contains basal medium plus 20 p.p.m. of penicillin G.

Table III. Effect of Presence of Living Plant Material on Growth Effect of Oxytetracycline

Treatment	Final Wet Weight, Mg.
Control	1390
Oxytetracycline, 5 p.p.m. (added at start)	2950
Oxytetracycline, 5 p.p.m. (added after 18 days)	2900

factor in the formation of stimulatory breakdown products of oxytetracycline.

Such results made it desirable to determine the effect of a longer growth period. Therefore, the group of antibiotics originally screened was rerun under identical conditions and allowed to grow for 8 weeks before wet weights were taken. The results are shown in Table IV.

Again, as in the 3-week growth period, there are considerable differences among the various antibiotics. For example, the two antibiotics (penicillin G and Bacitracin) which showed the least toxicity, the greatest stimulation, and the best stimulation at the highest level tested, showed similar results after 8 weeks, only more so. Compounds such as thiolutin and oxytetracycline, which caused slight stimulation at certain low levels at the end of 3 weeks, showed greatly increased stimulation at those same levels after 8 weeks. Streptomycin, which caused some stimulation at the lower levels and exerted no effect at the higher levels at the 3-week mark, showed no effect at the end of 8 weeks. Still another type of effect is shown by compounds like citrinin and patulin. These compounds are toxic above 1 p.p.m. and have little or no effect at that level after 3 weeks. At the end of 8 weeks, however, they show considerable stimulation at this low level.

Because degradation products of antibiotics such as oxytetracycline and chlortetracycline might have a role in their activity, and many of them are available as a result of work on the structure of oxytetracycline and chlortetracycline (*21*, *30*, *39*, *41*, *42*), a group of degradation products, as well as certain other derivatives, were tested to determine their effects on the growth of *Lemna*. The results showed four types of effect: stimulation, inhibition, no effect, and effect like that of the parent compound.

Structure seemed to offer no clue to stimulation of growth. Examination of other properties suggested that the chelation powers of these compounds might play a part in their activity. This hypothesis was not incompatible with the general results found by the workers at Boyce Thompson Institute in studying the effects of antibiotics on plant growth as measured by the wheat root test. They found that many antibiotics which stimulate the growth of this test system in distilled water fail to do so in tap water (*3*). Analysis of the tap water showed substantial amounts of trace elements, particularly iron. Subsequent work by the Boyce Thompson group with wheat roots and by the authors with *Lemna* showed that the chelating compound ethylenediaminetetraacetic acid (EDTA) or its sodium salts caused stimulation of both test plants as great as or greater than that caused by most of the antibiotics (Table V).

However, further tests with another group of oxytetracycline degradation products and other compounds, especially selected for their chelating ability, cast doubt that this idea provides the whole answer. Chelation plays a part in the growth of these plants, but where antibiotics fit into the chelation picture, if at all, is still obscure.

When plants from the 8-week penicillin G experiment were about to be weighed (Table IV), it was noticed that the control plants were starting to lose

Table IV. Comparative Effects of Antibiotics on Growth of *Lemna minor* Under Aseptic Conditions After 8 Weeks

Compound	% Change from Control on Wet Weight Basis			
	1 p.p.m.	5 p.p.m.	10 p.p.m.	20 p.p.m.
Actidione	− 70	Too much inhibition to measure		
Bacitracin	0[a]	+115[b]	+160[b]	+470[b]
Catenulin		Tissues disintegrated		
Chloromycetin	+ 10	+ 70	+ 50	− 65
Citrinin	+120	+100	− 20	− 98
Isonicotinic hydrazide	+ 25	+160	+210	+270
Neomycin	− 10	− 85	− 90	− 95
Netropsin	− 30	− 30	...	− 75
Oxytetracycline	+ 40	+350	+ 80	− 60
Patulin	+190	− 92	− 94	− 96
Penicillin G	+ 40[b]	+115[b]	+225[b]	+530[b]
Polymyxin	− 60	− 65	− 99	− 99
Rimocidin	0	0	0	+ 20
Streptomycin	0[a]	− 15[b]	+ 10[a]	+ 5[a]
Streptothricin	+ 10[a]	+ 25[a]	+110[b]	+265[b]
Thiolutin	+600[b]	− 97	− 98	− 98

Statistical analyses not carried out unless marked as follows:

[a] Not significant at 5% level.
[b] Significant at 1% level.

Table V. Effect of Ethylenediaminetetraacetic Acid on Growth of *Lemna*

P.P.M.	% of Control (Wet Weight)
10	+160
25	+275
50	+540
100	+125

Table VI. Effect of "Staled" *Lemna* Medium on Growth of Fresh Transfers of *Lemna*[a]

Treatment	% of Control (Wet Weight)
Aseptically removed	−60
Autoclaved	−65
Sintered-glass filtered	−80

[a] Test media made by adding equal parts of double-strength basal medium and "staled" medium.

their chlorophyll and had a poorer general appearance than a week before. The flasks containing 20 p.p.m. of penicillin G looked as if their growth curve had not leveled off; the controls looked as if their growth curve had leveled off and started back down. This suggested that some of the metabolic products formed by actively growing *Lemna* are secreted into the medium and are toxic. That this is true was shown by a group of experiments, the results of which are presented in Table VI. Apparently the toxic compounds produced are relatively heat-stable. Just when their concentration reaches the toxic point, and the role that antibiotics play in detoxification, have not been determined yet.

One point of discussion should be emphasized here: If antibiotics stimulate plant growth by neutralization of toxic effects, their general use in agriculture would be impractical, as leaching, diffusion, bacterial decomposition, adsorption by soil, and other causes would prevent the toxic products secreted by plants in nature from building up to effective levels. This mechanism of stimulation could account for discrepancies between in vitro positive results and field negative results, as in the flasks used for in vitro work the products pile up in a confined volume and the liquid medium constantly bathes the plant tissues.

Another important question which arises in the study of this problem is: How long do the antibiotics have to be in contact to be effective? Most of the data presented so far have been the result of terminal evaluations, weights, and measurements taken at the end of the test period for only one test condition. The importance of the time element, so prominent in the study of dynamic biological systems, yet so often overlooked (*2*), has not been considered thus far.

To study time relations, a large number of flasks with the basal liquid medium with 20 p.p.m. of pencillin G were inoculated with *Lemna* rosettes along with suitable controls. At 1- or 2-day intervals the *Lemna* contents of a group of five flasks with penicillin were transferred to flasks of basal medium without penicillin. Thus at the end of the experiment, there were sets representing exposure to "no penicillin," "penicillin for 2 days," "3 days," etc. Because of the size of this experiment and the length of time involved in its completion, the final results based on wet weights are not yet available. However, close observation shows no noticeable difference until exposure to penicillin G has taken place for at least 6 days. Here again, if the final data substantiate these preliminary observations, fundamental data will yield information of potential practical value. If a certain level of penicillin in solution must be maintained for a number of days before significant increases of growth can be obtained, practical application would not be justified.

Discussion

The present work has not answered the major question of how antibiotics act in affecting the growth of plants, but it has suggested some heretofore unconsidered possibilities and demonstrated again that stimulation of growth does exist. Because all this work as well as the previously presented tissue culture work (*27, 29*) was carried out under aseptic conditions, the role of microorganisms has not been considered.

Several hypotheses may be put forward as to the mechanism of action of antibiotic stimulation of plant growth: that degradation products formed after the antibiotics are in solution are responsible for the effect; that properties other than antibacterial properties are responsible for the effect—e.g., chelation; that the antibiotics do not actually affect the living cells directly, but cause a final increase in growth because of their detoxification of excreted toxic plant metabolic products; that permeability of the cell wall is affected, resulting in an increased water uptake or the initiation of water uptake sooner with seeds; that there is an interference with normal metabolism by either the antibiotic or one of its degradation products, such as the removal or inactivation of an inhibitor; that pathways of synthesis of essential limiting metabolites such as vitamins are affected in such a manner that greater synthesis is possible, even if only for a limited time; and that the hormone metabolism of the treated plant is affected.

Calvin and his coworkers (*16*) report that chlortetracycline and oxytetracycline accelerated photosynthesis in the alga *Scenedesmus* from two to six times, depending on the concentration employed. These two antibiotics also induced an abnormal increase in sucrose, which led these workers to suggest that "antibiotics could in some organisms have a direct accelerating effect on certain steps of metabolism."

At present the evidence does not clearly favor any one of these hypotheses. The answer might reside in a combination of hypotheses with a different combination for each antibiotic or at least for each physiological type of antibiotic.

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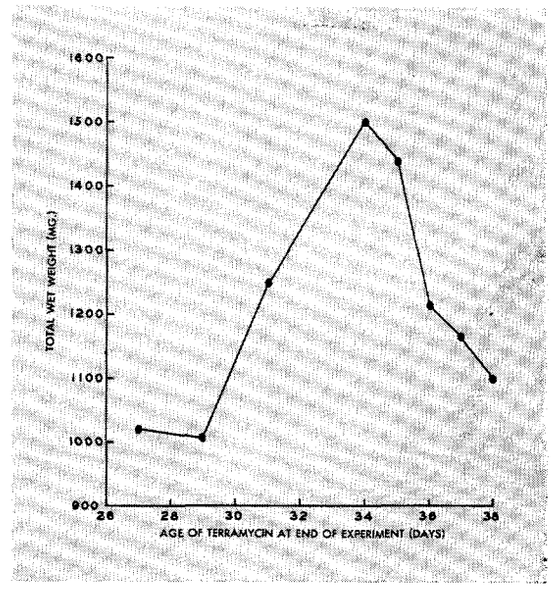
Figure 2. Effect of oxytetracycline of different ages in solution on growth of duckweed (*Lemna minor*)



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Received for review September 11, 1953. Accepted January 28, 1954. Presented before the Division of Agricultural and Food Chemistry, Fermentation Subdivision, Symposium on Nontherapeutic Uses for Antibiotics, at the 124th Meeting of the AMERICAN CHEMICAL SOCIETY, *Chicago, Ill.*

SOIL CONDITIONERS

Laboratory Evaluation of Polyelectrolytes As Soil Conditioners

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In the study of soil structure and its improvement with water-soluble polymers it was necessary to develop a method for comparing the soil-conditioning properties of many polymer samples in order to determine the effect of chemical composition, molecular weight, and molecular configuration. The method developed is a modification of a wet-sieve test for measuring aggregate stability. When used under closely controlled conditions the method is reproducible, is applicable to a variety of water-soluble polymers, and provides an index of the relative soil-conditioning activity of the polymer in use. The importance of a number of variables and their relationship to the test are discussed. The method is suitable for comparing different products as well as different production batches of a given product.

SYNTHETIC POLYELECTROLYTES are now an article of commerce as soil conditioners. In the manufacture of these materials it is necessary to be able to determine the relative soil-conditioning effectiveness of different chemicals and of different production batches of a given product. From the consumers' point of view, a method by which independent groups can compare the effectiveness of various commercial products is also desirable.

To answer the needs of the manufacturer and the consumer, a method for evaluating polyelectrolytes as soil conditioners should give reproducible results, should be applicable to a variety of polyelectrolytes, and should provide information on the activity to be expected when the products are used as soil-conditioning agents. Because polyelectrolytes function as soil conditioners by stabilizing soil aggregates, techniques that provide information on aggregate stability are the basis of many methods of evaluating polyelectrolytes.

In the past, several methods have been used to evaluate the effect of numerous additives upon soil structure. McCalla (*4*) used a pipet method to investigate