TABLE III. Effect of Hot Acid Treatment on Microbiological Assay of Urines.

Sample	γ/ml isonicotinyl hydrazine found	
	Before treatment	After treatment
1	48	45
2	13	53
3	31	44
4	5	40
5	10	21
6	6	28
7	11	61
8	19	17
9	25	19

24-hour pooled urine specimens, contributed by 9 normal human subjects who had taken orally 125 mg or 195 mg of isonicotinyl hydrazine. The acid-heat treatment is required to release the maximum anti-microbial activity from most urine specimens studied. Occasionally samples are encountered which do not require the acid-heat treatment, such as sample 1, and others are encountered (samples 8, 9) which, even after treatment, do not release all the anti-microbial activity indicated to be present by chemical assay. These data may be seen from Table III. The microbiological activity of aqueous solutions of isonicotinyl hydrazine is not altered by the acid-heat treatment.

Hydrazine has less than 2% the activity of isonicotinyl hydrazine in this assay and isonicotinic acid and isonicotinamide are completely inactive. Combinations of hydrazine and isonicotinic acid have no more activity than that of hydrazine alone. Drug-free urines, when treated as in the isonicotinyl hydrazine assay, have also been found to be completely inactive. Interference will be observed, however, if the urines contain streptomycin, dihydrostreptomycin, aureomycin, terramycin or chloromycetin. Paraamino salicylic acid causes no interference at a concentration of 1000 γ per ml and penicillin is without effect.

Further studies to increase the usefulness of the method are in progress.

Summary. A simple microbiological diffusion-plate assay for isonicotinyl hydrazine has been devised employing *Mycobacterium butyricum* ATCC No. 362 as the test organism. The method is suitable for assaying urines and other aqueous solutions containing 5 γ per ml or more of the drug. The solutions need not be sterile. The assay may be read in 18-24 hours and results are reproducible to ± 15% or closer. Results obtained by this method are in fair agreement with those obtained by the cyanogen bromide chemical assay.

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Stimulation of Plant Growth by Antibiotics. (19710)

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Preliminary experiments indicate that under appropriate conditions antibiotics can stimulate the growth of plant tissues, plant organs and whole plants. Although the extent of this phenomenon is at present unknown, tests are under way to determine which plants are affected and which antibiotics are effective. The present communication presents evidence for plant growth stimulation by antibiotics from 3 types of experiments.

Materials and methods. Tissue culture. The material used in these *in vitro* experiments was the virus tumor tissue from the root of the sorrel plant (*Rumex acetosa*).

The basal synthetic medium and the methods used in this type of experiment were the same as those previously described(1-3). Experiments were run in replicates of 5 and an average taken. *Seed germination.* The methods used for testing the effects of antibiotics on the growth of germinating seeds were similar to that described by Allen and Skoog(4). Seeds of the desired varieties were germinated on moistened filter paper in covered Petri plates containing 6 ml of water per plate. The desired concentration of the compound to be tested was included in aqueous solution; control plates contained only distilled water. The number of seeds per plate depended upon the size of the seed: with large seeds such as corn 5 seeds per plate were used, with smaller seeds such as sorrel 10 seeds were used. Germination took place at room temperature with the plates covered to exclude most of the light. Measurements were made at daily intervals after the emergence of the primary root. *Soil studies.* Greenhouse studies on seed germination and subsequent growth in soil were carried out in flats. Control flats were watered with tap water as the dryness indicated. Experimental flats were handled in exactly the same manner except that 5 ppm of the experimental antibiotic were included in the water.

Earlier experiments conducted in 1948 indicated that certain antibiotics in low concentrations stimulated the growth *in vitro* of virus tumor tissue from *Rumex acetosa*(5). The sterile technics employed eliminate the possibility that the stimulation observed is due to the effect of contaminating microorganisms. More recently it has been shown that to maintain satisfactory growth of this tissue vit. B_1 must be added to the medium used in subculturing(6). Its absence will result in death of the tissue after the second transfer.

Results and discussion. The addition of certain antibiotics to the basal medium at a concentration of 1 to 5 ppm in the absence of vit. B_1 will allow apparently normal growth of the virus tumor tissue. The results of this type of experiment are shown in Table I.

Germinating seeds of *Agave toumeyana* were stimulated in their growth by certain concentrations of thiolutin. The maximum effect was found to be at 1.0 to 5.0 ppm. The seeds germinated in the presence of 25 ppm of thiolutin had shorter roots than those treated with the solution containing 5 ppm, however, the coleoptiles were approximately the same length. In this experiment with thiolutin the concentrations ranged from 0.005 to 25 ppm; both sterilized and non-sterilized seeds were used with the same results.

TABLE I. Effect of Various Antibiotics on Growth of Virus Tumor Tissue from *Rumex acetosa.**

Antibiotic added	Concentration	Growth value
None (control)	—	1.3
Penicillin	10 u/ml	4
Terramycin	5 ppm	7.2
Streptomycin	5 "	5.3
Thiolutin	5 "	6.3
Bacitracin	5 "	10.9

* Experimental transfers grown in the absence of all vitamins for 2 weeks prior to experiment. Results are expressed in terms of growth value which is a number determined by dividing the final wet weight of the tissue by its initial wet weight.

TABLE II. Heights of Plants from Control Soil and Soil Treated with 5 ppm of Terramycin.

	Height in inches		
	High	Low	Avg
Sorrel			
Control	8.75	2.25	5.6
Treated	10.13	1.75	9.4
Sweet corn			
Control	19.5	6	14
Treated	24	9.75	17.45

The effects of terramycin on the germination and subsequent growth of sorrel seeds were tested in soil. It was observed that a greater percentage of seeds appeared above ground earlier in the treated flats than in the controls. This difference became more marked in their subsequent development as the plants approached maturity. The seedlings in the terramycin treated flats were definitely larger and more vigorous than those in the untreated flats. In order to determine if there would be a difference after the plants had become larger, these plants were allowed to remain in the flats with no further treatment for 46 days. At this time there were an equal number of plants in both the treated and control flats. The treated plants had an average of

5.2 leaves per plant whereas the control plants had 4.6 leaves per plant. The height of the plants from the ground level was measured. Table II gives the height of the tallest and shortest plants in each group, as well as the average height of all plants in each group in inches. The total weight of plant tissue above ground just as removed from the control flat was 60.9 g and from the treated flat 81.7 g. It is obvious from the foregoing results that the stimulatory effects of terramycin upon the germination and subsequent growth of sorrel plants caused a difference which was still apparent after 46 days.

A group of 49 sweet corn seeds was planted in each of 2 greenhouse flats: one control, one experimental. At the end of 4 weeks the plants were removed from the soil. Of the 49 seeds in the treated flat, 20 had germinated and formed a plant. Of those in the control flat, only 12 had germinated. The height of each plant from ground level was measured. Table II gives the height of the tallest and shortest plant in each group, as well as the average height of all plants in each group in inches. The total weight of plant tissue above ground just as removed from the control flat was 23 g and from the treated flat 45 g. The combined plant tissue from each group was dried at 105°C to a constant weight. The dry weight of all plants in the control group was 2.4 g and in the treated group it was 5.1 g.

Summary. Data from 3 types of experiments have shown a stimulation of plant growth by antibiotics: tissue culture, standard laboratory seed germination, and seed germination and subsequent growth in soil. The use of antibiotics in animal nutrition, particularly in the feeding of swine and poultry, is already prevalent. The preliminary experiments here described suggest the possibility that antibiotics might have a practical application in the stimulation of plant growth as well as in the control of plant diseases(7-9). The use of tissue culture technics should prove a useful tool in the study of the mechanism of stimulatory action by antibiotics.

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Intracellular Distribution of Estrogen Inactivating Mechanism in Rat Liver and Other Tissues.* (19711)

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The role of the liver in the inactivation of estrogens has been studied extensively for various species. A comprehensive review of the literature was presented by Paschkis and Rakoff(1). DeMeio and coworkers(2) and Coppedge *et al.*(3) demonstrated the importance of adding disphosphopyridine nucleotide (DNP) and nicotinamide when using male rat liver mince, as contrasted to liver slices, for the inactivation of alpha-estradiol. The object of the study here described was to

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