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Penicillin, Polymyxin, Thiolutin Control Infection in Beer Fermentation

CONTAMINATION INHIBITION

Antibiotics as Inhibitors of Microbiological Contamination in Beer

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The purpose of this investigation was to study the application of antibiotics to the control of bacterial and secondary yeast contamination in the brewery. Polymyxin was outstanding in its ability to control the Gram-negative bacterial infection of brewer's yeast and beer fermentation. The addition of bactericidal concentrations of polymyxin and other effective antibiotics to the fermenting beer results in a stimulation of the fermentation. Of the antibiotics studied, penicillin is the most effective in the control of the Gram-positive lactic acid bacteria in finished beer. Used in combination with thiolutin for the control of secondary yeast growth, it gives complete protection against microbiological growth in finished beer.

DANGER OF INFECTION with other microorganisms is one of the problems that are common to all microbiological processes, in which pure cultures are used. The fermentation of beer in a brewery is no exception and at times the bacterial infection of the fermentation presents a serious problem.

Brewer's yeast infected with bacteria to a dangerous extent is often treated with such weak bactericides as tartaric or phosphoric acid. This treatment, when carried out properly, kills a large percentage of the contaminating bacteria without affecting the yeast adversely. However, when such a treated yeast is used in a subsequent fermentation, the surviving bacteria again begin to multiply and the original level of infection is reached within a relatively short time.

The use of antibiotics in the control of bacterial infections of brewer's yeast was reported on by Gray and Kazin as early as 1946 (*1*). These investigators found that the antibiotic tyrothricin compared favorably with the commonly used bactericidal substances, but only when its concentration was raised to 500 p.p.m. Other investigators (*2, 3*) have reported that the growth of the lactic acid bacteria in finished beer can be controlled by the addition of penicillin.

Strandskov, Brescia, and Bockelmann (*5*) found that the antibiotic polymyxin inhibits the growth of the Gram-negative rods, Gram-positive lactic acid cocci, and Gram-positive lactic acid rods that commonly contaminate brewer's yeast and beer, at concentration levels that are not inhibitory to brewery yeast. These inhibitory studies were carried out on a yeast extract–dextrose medium.

These findings suggested that polymyxin as well as other antibiotics should be useful bacteriostatic and bactericidal agents in the brewery fermentation, during storage, and possibly in the finished product.

The effect of polymyxin on the Gram-negative bacterial infection during fermentation was studied using as fermenters 3-liter glass cylinders, 3 inches in diameter and 36 inches tall. It was demonstrated in a preliminary experiment that fermentation in such a cylinder proceeds at the same rate as in a plant fermenter. To test the effect on a fermentation, the desired quantity of an antibiotic, in 10 ml. of distilled water, was added to each cylinder except the control, to which 10 ml. of distilled water

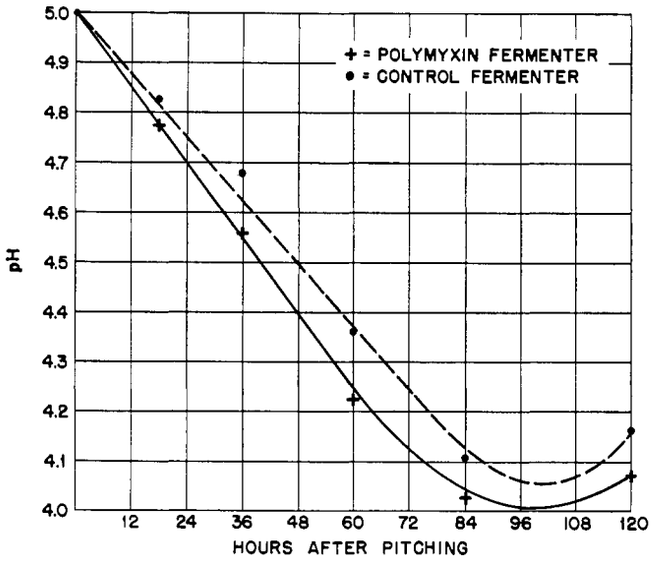


Figure 1. Effect of polymyxin on pH of fermenting beer

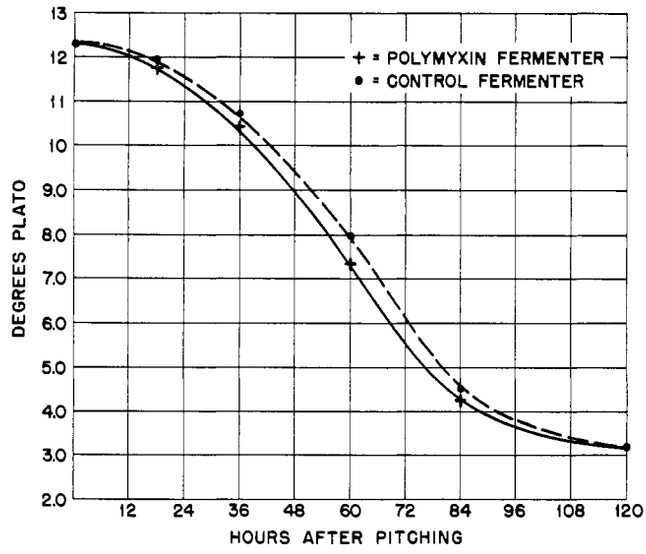


Figure 2. Effect of polymyxin on rate of beer fermentation

were added. Three liters of fermenting beer from a fermenter that had just been filled, which was contaminated with Gram-negative bacterial rods of the *Flavobacterium proteus* type, were added to each cylinder and the cylinders were suspended in a temperature-controlled water bath. The temperature of the bath water was adjusted twice daily, so that the fermentation temperature conformed as nearly as possible to plant fermentation temperatures. Aliquots of the fermenting beer were drawn daily for analysis. The bacterial plate counts were made on a dextrose–yeast extract medium which contained actidione as a yeast inhibitor (*4*).

Table I. Minimum Concentration of Antibiotics Bactericidal to Gram-Negative Rod Infection During Normal Fermentation

Antibiotic	Antibiotic Concn., γ/Ml.
Streptomycin	>125
Bacitracin	>125
Terramycin	50
Thiolutin	>10
Penicillin	>125
Subtilin	>125
Aureomycin	50
Polymyxin B.	0.005

The data in Table I show the minimum concentrations of the antibiotics studied that would completely eliminate the Gram-negative bacterial rod infection. This was the only type of infection present. Thiolutin is not very soluble in water and could, therefore, not be studied at a concentration level higher than 10γ per ml. of fermenting beer.

Terramycin, Aureomycin, and polymyxin were found to be the only effective antibiotics in the concentration levels studied. The activity of polymyxin was so superior relative to that of the other antibiotics that a rather detailed study of its effect on the fermentation was made.

Selected data are presented in Table II to show the effect of a wide range of polymyxin concentrations on beer fermentation. The bacterial infection is not affected by 0.001γ per ml., but is almost completely eliminated by 0.005 γ per ml. 48 hours after the start of the fermentation. Higher concentrations of the antibiotic are completely bactericidal to the Gram-negative rod infection. The fermentation is not adversely affected by polymyxin at concentration levels below 20γ per ml. The data in Table II indicate a possible stimulation of the fermentation by the antibiotic between the concentrations of 0.005 and 20γ per ml. as measured by the rate of reduction of pH and gravity of the fermenting beer. To verify this a number of fermentations were carried out in which the rate of fermentation of fermenting beers containing 0.05γ of polymyxin per ml. was compared with the fermentation rate of controls.

The data in Figures 1, 2, 3, and 4 are

Table II. Effect of Wide Range of Polymyxin Concentration on Beer Fermentation

Analysis	Polymyxin, γ/Ml.	Hours After Start of Fermentation 24	48	72	120
Yeast count × 10^{-6}	0.0	18.8	26.0	39.2	2.2
	0.001	18.0	29.3	37.3	2.6
	0.005	16.7	30.8	40.2	1.9
	0.05	20.2	30.5	44.9	2.4
	10.0	18.8	30.0	46.0	2.1
	20.0	19.5	29.7	39.3	4.3
	50.0	17.2	24.4	26.2	22.8
pH	0.0	4.73	4.48	4.26	4.18
	0.001	4.75	4.45	4.25	4.18
	0.005	4.73	4.42	4.13	4.09
	0.05	4.73	4.39	4.11	4.04
	10.0	4.72	4.38	4.10	4.04
	20.0	4.75	4.50	4.32	4.20
	50.0	4.78	4.68	4.56	4.32
Degrees Plato	0.0	10.90	8.31	5.27	3.08
	0.001	10.92	8.38	5.32	3.10
	0.005	10.95	8.34	5.28	3.09
	0.05	10.93	8.16	4.92	3.08
	10.0	10.80	8.12	4.96	3.10
	20.0	10.95	8.44	5.49	3.21
	50.0	11.10	9.96	8.79	5.58
Bacterial count, % of control	0.0	100	100	...	100
	0.001	100	100	...	100
	0.005	55	1.3	...	0.9
	0.05	1.2	0.0	...	0.0
	10.0	0.0	0.0	...	0.0
	20.0	0.0	0.0	...	0.0
	50.0	0.0	0.0	...	0.0

Table III. Effect of Polymyxin and Tartaric Acid Wash on Bacterial Count of Yeast and Fermenting Beer 0 and 24 Hours After Pitching with Washed Yeast

Type of Wash	Bacterial Count per Ml.		
	Yeast after wash	Fermenting Beer	
		0 hour	24 hours
Control	9,700,000	52,000	316,000
Tartaric acid	98,000	560	12,000
Polymyxin	140,000	480	0

Table IV. Effect of Several Antibiotics on Microbiological Contamination of Beer

Antibiotics Added	Condition of Beer in Bottles After 9 Weeks at 75° F.	Composition of Haze and Sediment
None (unpasteurized control)	Haze and heavy sediment	Secondary yeast + Gram-positive bacterial rods and cocci
Thiolutin (5γ/ml.)	Haze and light sediment	Gram-positive bacterial rods and cocci
Thiolutin, bacitracin (5γ/ml. each)	Haze and light sediment	Gram-positive bacterial rods and cocci
Thiolutin, subtilin (5γ/ml. each)	Haze and light sediment	Gram-positive bacterial rods and cocci
Thiolutin, streptomycin (5 γ/ml. each)	Haze and light sediment	Gram-positive bacterial rods and cocci
Thilutin, dihydrostreptomycin (5γ/ml. each)	Haze and light sediment	Gram-positive bacterial rods and cocci
Thiolutin, penicillin (5γ/ml. each)	Clear and very light sediment	Normal sediment
Thiolutin, terramycin (5γ/ml. each)	Haze and light sediment	Gram-positive bacterial rods and cocci
Thiolutin, polymyxin (5γ/ml. each)	Clear and very light sediment	Normal sediment
None (pasteurized control)	Clear and very light sediment	Normal sediment

The data in Figure 4 show that 0.05 γ of polymyxin does not affect yeast multiplication or the rate at which yeast settles out of suspension.

In all of the 12 fermentations to which 0.05γ of polymyxin was added, the bacterial count of the fermenting beers was reduced to zero during the first 48 hours of the fermentation.

The effectiveness of polymyxin as a yeast-washing agent was compared with that of the commonly used tartaric acid wash. A quantity of pitching yeast which was highly contaminated with Gram-negative rods of the *Flavobacterium proteus* type was divided into three batches. To one batch sufficient polymyxin was added to give a final concentration of 10γ of polymyxin per ml. of yeast slurry. The authors had found in the fermentation studies that this concentration of the antibiotic was not injurious to the yeast cells. To the second batch were added 2.6 grams of tartaric acid per 1000 ml. of yeast slurry. This lowered the pH of the yeast slurry to 2.9. This pH level has been found to give the maximum reduction of yeast bacterial contaminants without affecting the yeast adversely. The third batch of yeast was held as a control.

The three batches of yeast were then held at 45° F. for 4 hours before pitching in small 3-liter fermenters. Plate counts of the bacterial contamination of the

average results of analyses of 12 comparative fermentations.

The data in Figure 1 show that the pH of the fermenting beer, which contains 0.05γ of polymyxin per ml., decreases from the very beginning more rapidly than the pH of the beer in the control fermentation. The difference continues to increase until it reaches about 0.15 pH unit at approximately the 80th hour of the fermentation. Although the difference becomes less pronounced thereafter, the final pH value of the polymyxin-containing beer is approximately 0.1 pH unit lower than that of the beer from the control fermentation.

The effect of polymyxin on the rate of decrease in gravity of the fermenting beers is presented in Figure 2. The rate of fermentation, as measured in this manner, is also stimulated by polymyxin, but the final gravity of the beers is not affected.

In Figure 3 the pH of the fermenting beer is plotted against the fermenting extract. The data in this figure show that the rate of pH reduction of the fermenting beer is affected to a greater extent than is the rate of extract fermented. At any selected degree of fermented extract the pH is much lower in the polymyxin-treated fermentation than in the control.

Figure 3. Effect of polymyxin on relationship of pH to extract fermented

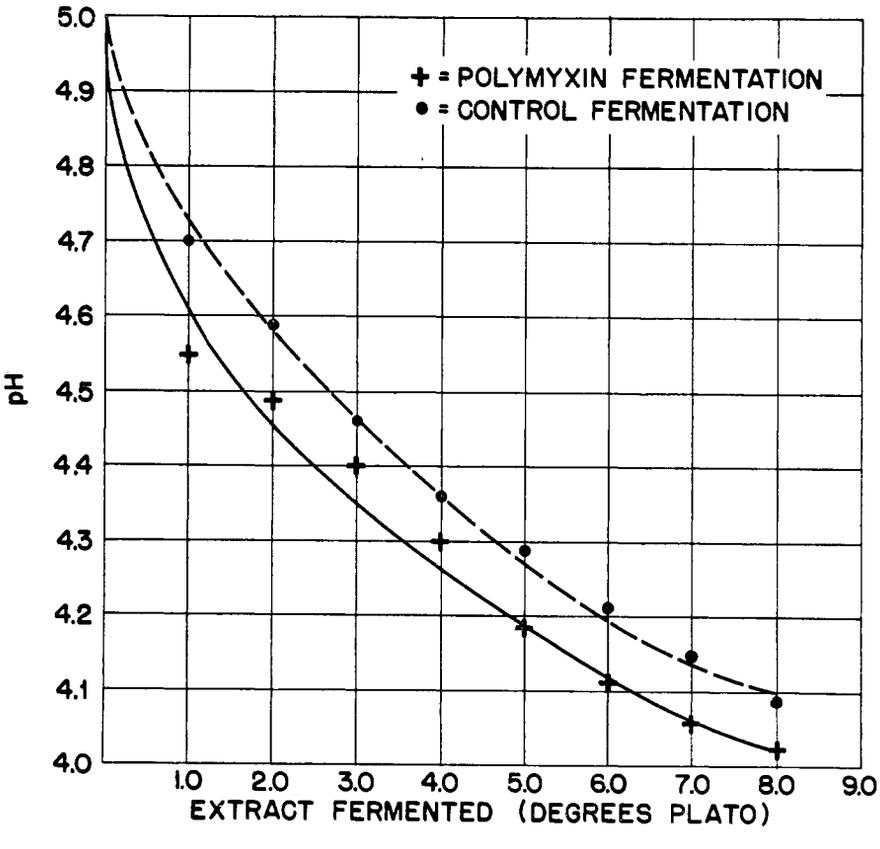


Table V. Concentrations of Polymyxin, Pencillin, and Thiolutin Required to Inhibit Growth of Microbiological Contaminants in Beer

Polymyxin, γ/Ml.	Penicillin, γ/Ml.	Thiolutin, γ/Ml.	Condition of Beer After 9 Weeks at 75° F.	Composition of Haze and Sediment
0[a]	0	0	Haze and heavy sediment	Gram-positive bacterial rods and cocci + secondary yeast
3.0	0	3.0	Clear and very light sediment	Normal sediment
1.0	0	3.0	Clear and very light sediment	Normal sediment
0.3	0	3.0	Clear and light sediment	Gram-positive bacterial rods
0.1	0	3.0	Clear and light sediment	Gram-positive bacterial rods and cocci
0.03	0	3.0	Clear and light sediment	Gram-positive bacterial rods and cocci
0	0.1	3.0	Clear and very light sediment	Normal sediment
0	0.03	3.0	Clear and very light sediment	Normal sediment
0	0.01	3.0	Clear and light sediment	Gram-positive bacterial rods
0	0.003	3.0	Hazy and light sediment	Gram-positive bacterial rods
0	0.001	3.0	Hazy and light sediment	Gram-positive bacterial rods and cocci
0	1.0	3.0	Clear and very light sediment	Normal sediment
0	1.0	1.0	Clear and very light sediment	Normal sediment
0	1.0	0.3	Haze and heavy sediment	Secondary yeast
0	1.0	0.1	Haze and heavy sediment	Secondary yeast
0[b]	0	0	Clear and very light sediment	Normal sediment

[a] Unpasteurized control.
[b] Pasteurized control.

yeast were made on the original yeast, the three yeast batches at the end of the 4-hour holding period, and the fermenting beer 24 hours after the start of fermentation.

The data in Table III show that tartaric acid and polymyxin reduce the viable bacterial contaminants to approximately the same extent during the 4-hour holding period. However, the surviving bacteria in the fermenter pitched with the tartaric acid–washed yeast grew rapidly during the fermentation, as evidenced by the 24-hour plate count. The polymyxin, on the other hand, continued to exert a bactericidal action in the fermenter and after 24 hours the bacterial infection had been completely eliminated.

The effect of antibiotics on microbiological growth in finished beer was also investigated. To do this, the antibiotic to be tested was added in 1 ml. of sterile distilled water to clean empty 12-ounce bottles. The bottles were put on the regular production line, where they were filled with beer and crowned. The filled bottles were incubated at 75° F. and were observed weekly for the development of sediment and turbidity. Pasteurized and unpasteurized bottles of beer were also incubated as controls.

In order to test the effectiveness of antibiotics as inhibitors of bacterial growth in beer, it was necessary to find an agent that would inhibit the secondary yeast growth that is usually obtained. Four fungistatic antibiotics including actidione, rimocidin, nystatin, and thiolutin were investigated. Of these, thiolutin was found to be the most effective in the control of secondary yeast growth in finished beer and was used, for this purpose, in an experiment in which the effectiveness of several antibiotics as bacteriostatic agents was tested.

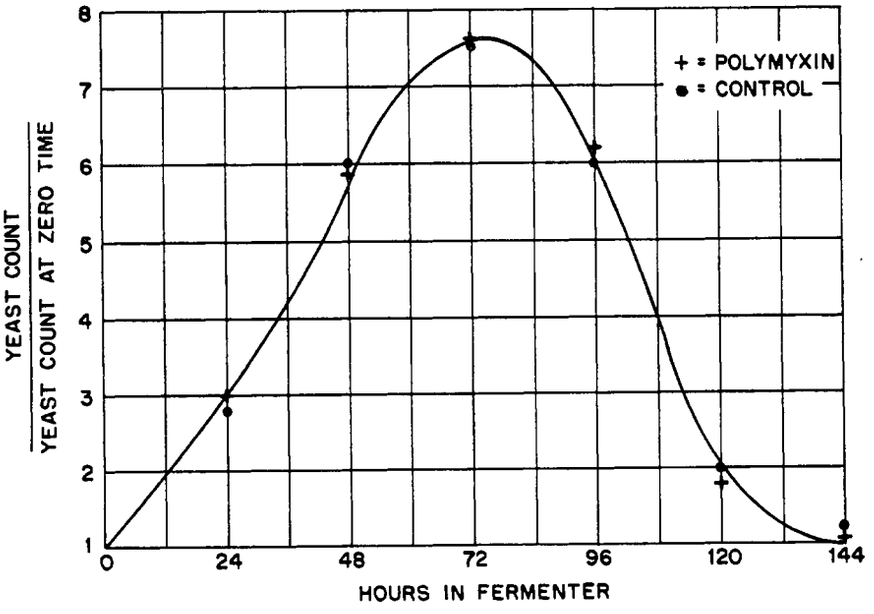
The data in Table IV show the effectiveness of 5γ per ml. of several antibiotics, in combination with 5γ per ml. of thiolutin, in the control of bacterial growth in finished beer. At the concentration level studied only penicillin and polymyxin in combination with thiolutin completely eliminated the growth of microorganisms in finished unpasteurized beer.

The minimum effective concentrations of these three antibiotics were determined (Table V). Penicillin is considerably more active than polymyxin in the control of the Gram-positive bacterial contaminants and in each case the concentration of antibiotic required to inhibit the growth of the Gram-positive cocci is lower than the inhibitory concentration for the Gram-positive rods. Thiolutin inhibits the secondary yeast growth in unpasteurized beer at a concentration level of 1.0γ per ml.

Discussion

The effectiveness of antibiotics, at these trace concentration levels, in the control of microbiological contaminants of brewer's yeast and fermentation should be of practical value to the brewing industry. The bactericidal agents, such as tartaric and phosphoric acid, which are commonly used for the treatment of contaminated brewer's yeast do not completely eliminate the bacterial contamination. Furthermore, the bactericidal action of these agents is terminated as soon as the yeast is pitched, as the bactericidal action is to a large extent a result of the pH obtained by addition to the pitching yeast slurry. The bactericidal action of the antibiotics is not the result of such a secondary effect and, as demonstrated with polymyxin in the present investigation, they continue to exert a bactericidal action during the fermentation. Actually, as no holding time is required, the antibiotic can be added to the yeast just before pitching. The antibiotic can also be added directly to the fermenter or to the wort as it is being pumped to the fermenter. It is important, however, that it be thoroughly blended with the beer at the start of the fermentation.

Figure 4. Effect of polymyxin on yeast growth and yeast in suspension during fermentation



Although polymyxin was found to be the most effective antibiotic, of those studied, for the control of the Gram-negative bacteria that infect yeast and fermentations, it is entirely possible that other antibiotics will be superior to polymyxin in the control of the lactic acid rods and cocci that infect brewer's yeast and fermentations. For example, penicillin is more active than polymyxin against the lactic acid bacteria in finished unpasteurized beer and a combination of antibiotics such as penicillin and polymyxin may be required for the complete control of bacterial contaminants of brewer's yeast. It was, however, surprising to find that polymyxin is more active against the Gram-positive lactic acid bacteria in finished beer than any of the other antibiotics tested, with the exception of penicillin. Polymyxin is generally considered as being specific for Gram-negative bacteria. A more thorough study of its activity in beer may help to elucidate its mode of action.

The stimulation of the fermentation by polymyxin is interesting. The same type of stimulation was observed when a bactericidal concentration of terramycin and aureomycin was added to the fermentation. It seems possible, therefore, that this stimulation is related to the elimination of bacterial action during the fermentation and may not be a direct effect of the antibiotic on the yeast.

Acknowledgment

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NOTES AND COMMUNICATIONS

Weeds Contain Valuable Chemical Compounds

As a result of our increasing population and rising standard of living it becomes economically important to investigate the chemical possibilities of some noneconomic plants that can be grown readily and in good yield. Results of analyses, by acceptable methods, of representative samples of several such plants are listed in Table I. These data indicate that smartweed, poverty grass, sedge, and the stalk of the Jamestown weed have industrial possibilities on the basis of their high content of furfural, cellulose, or α-cellulose. Purslane, for example, is especially rich in pectic compounds. Noneconomic plants may have an important role in meeting soil and economic conditions in the future.

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Table I. A Partial Chemical Analysis of Several Noneconomic Plants

(On an ash- and moisture-free basis)

Plant	Total Soluble Sugars, %	Total Pectic Compounds, %	Total Furfural, %	Cellulose %	α-Cellulose %
Smartweed, *Polygonum hydropiper* L.	5.32	6.33	8.18	42.11	72.20
Nettle, *Urtica dioica* L.	4.33	2.98	7.23	25.42	67.13
Jamestown weed (stalk), *Datura stramonium* L.	7.28	7.99	11.55	46.13	65.40
Poverty grass, *Aristida dichotoma* Michx.	2.86	Trace	15.25	49.76	61.89
Milkweed, *Asclepias syriaca* L.					
Leaves	4.60	1.27	5.54	30.26	59.47
Stalk	11.62	1.79	6.85	31.79	...
Curled dock, *Rumex crispus* L.	6.90	2.17	6.33	30.93	64.44
Wild lettuce, *Lactuca scariola* L.					
Leaves	4.40	2.88	5.26	19.29	66.83
Stalk	14.65	1.16	8.95	38.23	68.16
St. John's-wort, *Hypericum perforatum* L.	7.60	1.44	7.98	39.14	59.53
Lambs quarter, *Chenopodium album* L.	0.85	5.00	7.85	32.27	56.96
Butterweed, *Erigeron canadensis* L.					
Leaves	2.74	2.12	4.74	16.88	71.47
Stalk	4.90	3.43	11.32	49.09	64.83
Purslane, *Portulaca oleracea* L.	2.91	9.14	6.63	25.01	...
Couch grass, *Agropyron repens* (L.) Beauv.	8.75	0.55	11.95	42.34	62.56
Golden rod, *Solidago rugosa* Ait.	2.62	4.65	8.49	37.72	64.87
Aster, *Aster* sps.	4.17	3.22	10.18	44.29	67.86
Sedge, *Scirpus atrovinens* Willd.	10.75	1.33	11.54	43.93	65.85
Catnip, *Nepeta cataria* L.	6.17	4.44	8.98	40.25	66.27
Wild carrot, *Daucus carota* L.	8.75	3.50	8.88	38.31	65.40
Flagroot, *Acorus calamus* L.	7.32	3.40	7.81	30.87	62.37
Joe-Pye weed, *Eupatorium maculatum* L.	5.62	2.88	7.00	23.22	63.91
Yarrow, *Archillea millefolium* L.	3.59	0.87	9.73	33.62	64.23
Sumac, *Rhus typhina* L.					
Leaves	7.50	2.35	4.10	20.23	52.69
Blossom	5.62	2.21	9.79	31.00	62.07
Hardhack, *Spiraea tomentosa* L.	3.02	2.00	7.67	29.54	...
Dock, *Arctium minus* (Hill.) Bernh.					
Leaves	3.90	1.69	5.45	21.72	...
Stalk	11.43	1.18	9.03	36.74	...
Ragweed, *Ambrosia artemisiifolia* L.	2.80	3.95	6.46	23.61	65.40

Water Culture Crops Designed to Study Deficiencies in Animals

SIR: A paper by McClendon and Gershon-Cohen (*2*) appearing in a recent issue of this journal contains a number of statements that merit comment.

To the best of our knowledge, this is the first attempt to produce feeds for special nutrition studies by means of hydroponics. As such, the paper is of considerable interest to everyone working in the field of mineral metabolism.

The authors implied that they produced a fluorine deficiency in rats fed plants grown according to their directions. The "fluorine-free" ration contained 30% yellow corn, 30% sunflower seed, 20% sunflower leaves, 10% yeast, and 10% sucrose. The corn and sunflowers fed this group were raised in water. The "control" animals received a similar diet, with the corn and sunflowers grown in soil. The drinking water for the latter group contained 20 p.p.m. of fluorine. A basic tenet of the scientific method requires that the control rats be fed plant materials grown under exactly the same conditions as those used for the mineral-"free" group plus the mineral being studied. If that had been done, then any growth stimulation resulting from the addition of pure fluorides would have been good evidence that the diet contained less of this element than that required by the rat.