Citrinin as an Antibiotic

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This is a preliminary report on bacteriologic, toxicologic, and therapeutic studies on citrinin begun by two of us in the Pincomb Chemical Research Laboratories in the early spring of 1945 and participated in by the group in The National Institute of Biological and Chemical Production beginning in 1947. The citrinin used in these experiments was isolated from the culture medium of *Penicillium citrinum* Thom, identified by G. Smith in England through the courtesy of J. Needham and found to be identical to that of Hetherington and Raistrick (*2*).

The study on bacteriostatic properties of citrinin was begun before V-E Day in complete ignorance of the work of Raistrick and Smith (*4*), Robinson (*5*), Oxford (*3*), and Ambrose and DeEds (*1*). The details of all these reports on antibiotic properties, with the exception of that appearing in the *Journal of Pharmacology*, are still not accessible to the authors. Table 1 summarizes the results from our inhibition experiments with citrinin on bacterial growth.

TABLE 1

Species of bacteria	Gram stain	Minimum concentration of citrinin (mg./cc.)	Results	
Str. viridans	+	0.05	Complete	inhibition
B. mycoides	+	0.05–0.1	"	"
B. graveolens	+	0.1	"	"
Staph. aureus	+	0.4	"	"
Pneumococcus	+	0.5	"	"
V. cholerae	−	1	"	"
B. typhosus	−	1	"	"
B. dysenteriae Flexner	−	2–4	"	"
B. coli	−	4	"	"
B. paratyphosus	−	8	"	"
Ps. pyocyanea	−	>15	Partial	"

For inhibition tests, 1 drop ot a 24-hour bacterial culture was added to 1 cc. of ordinary broth medium containing different amounts of citrinin. After incubation for 16 hours, readings were taken. The figures in the third column represent the minimum concentrations of citrinin required for the complete inhibition of growth of different species of bacteria within 16 hours of incubation. It is evident that gram-positive bacteria are more susceptible to citrinin than gram-negative ones. *Streptococcus* and *Bacillus mycoides* are the most susceptible.

It is interesting to note that bacteria could be "sensitized" by citrinin so that their resistance was significantly reduced by continuous subcultivations in media containing subminimal concentrations of citrinin. For instance, untreated *Staphylococcus aureus* required 0.4 mg./cc. of citrinin for complete inhibition, but after 10 treatments with citrinin (2 × 0.1 mg., 8 × 0.05 mg./cc.) it required only 0.05 mg., the time interval between two subsequent treatments being always 24 hours. The bacteria so treated resumed their normal multiplications immediately after being transferred into an ordinary broth medium. After 9 transfers in the broth, the descendants were still sensitive to citrinin, since 0.1 mg. of citrinin was already sufficient for their complete inhibition. Another instance is *Pseudomonas pyocyanea*. This could be inhibited by 4 mg. of citrinin after 9 subsequent subcultivations in a 0.4 per cent citrinin-broth medium, which was normally ineffective. The other bacteria (*Str. viridans*, *B. typhosus*, *B. dysenteriae*, *B. coli*, *B. paratyphosus*, and *B. mycoides*) behaved similarly.

Another remarkable finding was that the citrinin-treated staphylococci were also more susceptible to sulfadiazine. For example, those which were untreated required more than 5 mg. of sulfadiazine/cc. of medium for inhibition, while the "sensitized" ones needed 1 mg./cc. only.

Human serum (10 per cent) had no significant effect on the action of citrinin on *Staphylococcus*, *B. typhosus*, and *B. mycoides*. Cysteine (0.25 per cent) had no counteraction on citrinin. Sodium thiosulfate (0.5 per cent) and p-aminobenzoic acid (0.1 per cent) had no antagonistic action.

For toxicity tests we used albino rats and rabbits as experimental animals. For the rat the intraperitoneal lethal dose of citrinin was 1.7 mg./100 grams body weight, and for the rabbit, 5. mg. Autopsy showed hemorrhages in lungs and liver and accumulation of blood in the chest cavity. Repeated daily injections of citrinin in increasing doses resulted in increasing the tolerance of the animal to this substance. One rat (350 grams) was still living after receiving a total amount of about 155 mg. in 27 injections (1 × 0.8, 5 × 2.8, 8 × 4, 5 × 6, 4 × 8, and 4 × 12 mg.). A single intravenous injection of citrinin (2 mg./100 grams body weight) into the rabbit caused lacrimation, nasal discharge, salivation, drowsiness, and lowering of body temperature.

For therapeutic tests we have tried local application of citrinin on rabbits and human beings, and the results seemed very encouraging. In one experiment four rabbits of about 2 kg. were used. A piece of skin (1 × 3 cm.), together with some muscle beneath, was excised and the wound inoculated with *Staph. aureus*. After extensive pus formation two of the animals were treated locally with citrinin powder, one with sulfadiazine powder, the fourth, without any treatment, serving as a control. The citrinin-treated animals responded excellently. On the following day the lesions dried up, crusts formed, and in two days the wounds had healed. The sulfadiazine-treated rabbit showed some improvement, although some pus was still present on the fourth day of treatment. The control showed no improvement at all within 7 days.

Clinically, citrinin has been used in three cases of local infections, all of which showed dramatic results. The first had a cheek carbuncle with an indurated base inches in diameter; the second, an infected wound in the right palm; and the third, an ulceration with marked redness and induration over the chin and with enlarged and tender submaxillary lymph glands. The first two were staphylococcal infections; the last, streptococcal. Citrinin-sodium bicarbonate powder was applied locally to the infected region, and the indurated base was infiltrated with sodium-citrinin solution (1 per cent), after which it was dressed with citrinin-soaked gauze. At first, all experienced some needling pain but no other discomfort. The infected area dried up in 6–18 hours, the crust formation being followed by rapid healing.

The study on citrinin is still being carried on in the Pincomb Chemical Works along chemical, bacteriological, and clinical lines. The details of the above experiments will be published elsewhere.

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Lactobacillus acidophilus Counts in the Saliva of Children Drinking Artificially Fluorinated and Fluorine-free Communal Waters[1]

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It has been demonstrated that there is a direct correlation between the number of *L. acidophilus* organisms in the saliva and dental caries activity in the mouth (*3*). In mouths with active dental decay the number of bacteria per cubic centimeter of saliva is high (20,000 or more), and in mouths in which the teeth are immune to decay at any specific time the counts are negative (under 100). Studies conducted by Jay (*5*) among representative population groups reveal that approximately 15 per cent of the individuals sampled have negative counts and about 57 per cent significantly high counts.

It has been established by Dean, *et al.* (*4*) that in areas in which fluorine occurs naturally in the drinking water supply there is a lower-than-expected incidence of dental decay among the group of the population studied (12- to 14-year-olds). In these fluoride areas, there is a much-greater-than-expected proportion of negative *Lactobacillus* counts (37.4 per cent) and a smaller-than-expected proportion of high counts (27.5 per cent) when the drinking water contains more than 1 ppm fluorine. The results of these *Lactobacillus* studies tend to confirm the dental findings.

[1] The media were furnished by the Division of Laboratories and Research, New York State Department of Health.

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The purpose of this paper is to present a preliminary observation made in connection with the studies of *L. acidophilus* counts in the Newburgh-Kingston Fluorine Demonstration, now being conducted by the New York State Department of Health (*1, 2*).

In order to determine the efficacy of introducing fluorine artificially into a public water supply for the purpose of reducing the incidence of dental caries, 1 ppm fluorine as sodium fluoride has been incorporated into the Newburgh water supply continuously since May 1945. Kingston, a comparable city, is consuming fluorine-free water and is being used for control purposes.

In Newburgh, individual *Lactobacillus* counts were made on random samples of the school population in 1944 and again in 1946 and 1947. The Kingston school population was sampled in 1946 and 1947. The findings are depicted in Fig. 1.

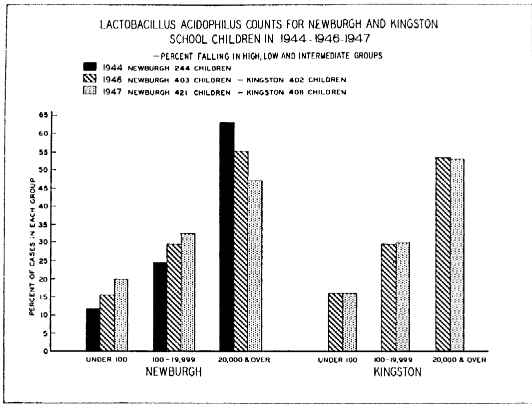

FIG. 1. *Lactobacillus acidophilus* counts for Newburgh and Kingston school children 1944–1946–1947—per cent falling in high, low, and intermediate groups.

Samples of saliva collected from 244 children of school age in Newburgh late in 1944 (prior to the introduction of fluorine into the municipal water supply), showed that 11.9 per cent were negative, and 63.5 per cent had counts of 20,000 or over. Analyses of salivas of 403 children in the same community early in 1946 revealed that there were 15.4 per cent negative counts and 55.2 per cent high counts. Of 421 samples collected early in 1947, 20 per cent had negative counts, while 47.3 per cent had high counts. In contrast, an analysis of the salivas of 402 children in Kingston early in 1946 revealed that 16.2 per cent had negative counts and 54.2 per cent, counts of 20,000 or over. In early 1947, among 408 children in Kingston, 16.2 per cent had negative counts and 53.9 per cent, counts of 20,000 or over.

It will be observed that while in Kingston the percentages of high and low counts remained approximately constant for the two-year period, there has been a consistent drop in percentages of high counts and a rise in the percentages of low counts in Newburgh since fluorination of the municipal water supply.

The standard significance tests were applied to the differences between the 1944 and the 1947 figures. The rise in the percentage of low counts (8.1 per cent) was 2.68 times its standard error, with a chance probability of .007. The drop in the percentage of high counts (16.2 per cent) was 4.03 times its standard error, with a chance probability of .00006. These differences may be considered statistically significant.