sought contained, among others, strains from several epidemics of septic sore throat which occurred in Massachusetts and Connecticut within recent years. Therefore it was logical to search for the desired phage in sewage from cities of those states during the season when streptococcus infections are prevalent. I am indebted to the health officers of Boston and Hartford for the two samples of sewage examined. Both were received in January, 1934. The examination of the two samples was made simultaneously.

TECHNIQUE

The technique was similar to that commonly employed to obtain from sewage phages active against other species of bacteria. The medium was meat infusion broth of double strength, which was distributed in test tubes, sterilized by heat and then diluted with an equal quantity of sewage from which all bacteria were removed by filtration. To this medium 25 per cent. of sterile filtrate was added from cultures as described below.

For the first culture generation of streptococci the filtrate added to the sewage medium was from culture planted with the unfiltered sewage, and incubated overnight at 37° C. The inoculum for this first culture generation of streptococci was with a mixture of 10 strains. A very light inoculum was prepared by adding one drop of over-night culture of each of the strains to a tube containing about 10 cubic centimeters of broth, and planting one drop of this diluted mixed culture in the sewage medium. After growth over night the culture was filtered, and the filtrate was tested to demonstrate any lytic principle it might contain by planting each of the 10 strains in pure culture into broth containing 10 per cent. of the filtrate.

For the second and following culture generations in the sewage medium inoculations were made with the 10 strains in pure culture, while the filtrate added to the medium was a mixture of filtrates from the 10 preceding pure streptococcus cultures. The lot of 10 streptococci was made up of representative strains from the group for which a new phage was sought.

A weak lytic principle appeared in the filtrate of the third culture generation in the medium containing the Boston sewage. The procedure was continued, using now only 3 or 4 of the most sensitive streptococcus strains of the lot. It required only a few more such passages to attain what appears to be the maximum potency for this race of phage—the ability to lyse sensitive strains in a dilution of about 1 to 10^6.

This new race, designated the "Boston" phage, is not neutralized by any one of the 3 serums specific for the Wisconsin, Michigan or Cincinnati races of phage. (Anti-phage serum is prepared by treating rabbits repeatedly with the phage for which the antibody is desired.) Hence there are now 4 antigenically distinct races of streptococcus bacteriophage available for study.

Both the Boston and the Hartford samples of sewage were further examined for the presence of a phage similar to the Cincinnati race. The procedure was as outlined above, except that the 10 selected strains of streptococci were all sensitive to the Cincinnati phage. A lytic principle appeared in the filtrates from the first generation of the mixed streptococcus culture in both the Boston and Hartford media. Both lysates were active against the same strains of streptococci as the Cincinnati phage, and both were neutralized by the serum of a rabbit treated with the Cincinnati phage. According to these criteria both races are identical with the Cincinnati phage.

The readiness with which bacteriophage was obtained from the Boston and Hartford samples of sewage indicates that streptococcus bacteriophage is widely distributed, at least during the season when streptococcus infections are prevalent.

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EFFECTS OF PROTRACTED EXPOSURE TO A LOUD TONE[1]

WITHIN recent years, Yoshii and others have published evidence which purports to show that protracted exposure of animals to intense tonal stimulation results in localized cochlear lesions whose foci are dependent upon the exposure-frequencies.[2] More recently, Upton attempted to determine the functional effects of exposing guinea pigs to a 1,000-cycle tone, and reported marked loss of acuity to the exposure frequency but no loss at 500 cycles.[3] These results have been widely accepted and are commonly interpreted in favor of the resonance-hypothesis. The methods employed do not always carry conviction, however, particularly in the crucial matter of measuring what the animal actually can, and can not, hear. The indicators by which the animal reveals that it hears a given tone (usually some form of startle-behavior, *e.g.*, pinna- or breathing-reflex) are notoriously subject to extinction by the very condition of long-continued exposure to that tone; hence we

[1] Communication No. 5 from the Alpha Research Laboratory, Department of Psychology, established and maintained with aid from the Research Trustees, American Otological Society. Special aid in this problem from the Elizabeth Thompson Science Fund is gratefully acknowledged.

[2] For references, see C. v. Eicken, *Abderhalden's Handb. d. biol. Arbeitsmethoden*, v: 771, 1929.

[3] M. Upton, "Functional Disturbances of Hearing in Guinea Pigs after Long Exposure to an Intense Tone," *Jour. General Psychol.*, 2: 397–412, 1929.

can seldom be sure whether animal's failure to respond is due to actual inability to *hear* that tone or due merely to extinction of the overt response. We have long been convinced that this problem, whose importance for questions of general auditory theory is commonly admitted, merits systematic re-examination; and the results hitherto obtained tend to confirm this conviction.

The actual acuity of a female dog A was measured by an established form of the conditioning procedure[4] at nine separate frequencies from 200 to 5,000 cycles. She was then exposed to a continuous 1,000-cycle tone (whose energy-level was about 110 decibels above her limen) for 18 hours within the space of five days. Her acuity was then found to have fallen by forty to fifty decibels, not at 1,000-cycles alone but throughout the entire test-range (Table I, row 2). Her limens remained virtually the same, despite continuance of the exposure, for an additional period of 8 days. Then, in hope of achieving still greater loss, we *interrupted* the exposure-tone 52 times a minute (each burst of sound continuing about 0.12 second). After 15 hours of this intermitted sound within the course of a single day, hearing was further reduced about thirty decibels (row 3), whereupon it again remained virtually level, except for a slow recession. When 148 hours of interrupted stimulation (distributed through 11 days) had been completed, all exposure was terminated. The animal was soon thereafter dispatched and preliminary steps for histological examination begun.

TABLE I

(1) Frequency ...	200	500	800	900	1000	1100	1200	2000	5000
(2) Loss in decibels after 81 hours continuous tone	49	38	52	46	48	48	49	36	53
(3) Total loss in db. after 148 hours interrupted tone	71	80	85	80	81	84	83	76	83

To make sure that this great reduction was not due in whole or in part to mere extinction of the conditioned response (flexion of right foreleg), whereby we test animal's ability to hear, we placed dog B under *complete general anesthesia* (Nembutal) during each exposure-time of ten hours. Inasmuch as the animal "slept" peacefully through the whole exposure, the response-mechanisms which had been conditioned into the higher neural centers were presumably shielded from disruption, whereas the peripheral acoustic apparatus was being affected much as usual. An interrupted tone of 3,000-cycles (intensity about 110 db. above B's limen) was used, and exposure continued for five 10-hour stages. Table II shows the loss in acuity after each of these 10-hour periods.

[4] For description of training and testing technique, see SCIENCE, 78: 269–270, 1933.

TABLE II

Cumulative exposure	Test frequencies							
hours	500	1000	2000	2500	3000	3500	4000	5000
10	33	20	40	32	33	30	25	17
20	46	47	59	60	55	62	59	55
30	67	56	70	71	70	71	75	72
40	59	49	74	79	73	78	78	71
50	56	47	77	79	72	78	78	71

Thirty-one days after the last exposure, B still shows no signs of recovery from the levels reached directly after the final exposure.

Several conclusions seem to emerge from these observations:

(1) Protracted stimulation by loud tones of medium pitch (1,000 and 3,000 cycles) induces an almost *horizontal subsidence* in auditory acuity throughout an extensive section of the audible range.

(2) No sign of functional restoration appears as much as thirty-one days after the last exposure.

(3) The *interrupted tone* proves to be an extremely effective instrument for acoustic impairment. It is well known that a steel bar, when exposed to repeated stresses of appropriate force, fails sooner than under a dead load of the same magnitude; analogous principles may be operative here. Certainly these recurrent bursts of sound are more distressing to the human ear than is a continuous tone of the same amplitude.

Until histological examination is effected, any theoretical inquiry would be premature. Several explanatory possibilities may be noted: (1) Wide-spread cochlear lesions; (2) middle ear effects, such as spastic degeneration of the tensor tympani; (3) even coagulation of proteins in the cochlear perilymph might be suggested.

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