antibody and complement in vivo. Whether these complexes of surface antigens, antibody, and complement act to suppress or to accelerate lymphocyte cytotoxicity and complement-dependent cytolysis of tumor cells in vivo under particular conditions is not yet known. However, B (bone marrow derived) lymphocytes and neutrophils that have receptors for cell-bound C3 (*8*) may react to C3 complexed on tumor cells and play an important role in the interaction of tumor and lymphoid cells. This interaction would be a significant factor for prognosis in the cancer patient.

The above-described IA techniques should be applicable to immunological studies of human autoimmune diseases such as certain types of thyroiditis and glomerulonephritis as well as to cancer studies.

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Interspecies Conversion of Clostridium botulinum Type C to Clostridium novyi Type A by Bacteriophage

Abstract. *When* Clostridium botulinum *type C is cured of its prophage it simultaneously ceases to produce toxin. This nontoxigenic culture can then be converted to another toxigenic bacterial species,* Clostridium novyi *type A or to toxigenic* Clostridium botulinum *types C or D, by specific bacteriophages. The toxigenicity and type of toxin produced by these cultures depends upon the continued presence of these bacteriophages.*

Clostridium botulinum and *Clostridium novyi* are pathogenic anaerobes that are characterized by their ability to produce powerful toxins. The *C. botulinum* group produce neuroparalytic toxins that are responsible for botulism in man and animals. Wound botulism has been reported in man, but botulism usually occurs in both man and animals from the ingestion of food containing toxin produced by one of the several known types of *C. botulinum*. *Clostridium novyi* also produces lethal toxins; it is often found in gas gangrene infections of man and animals and causes necrotic hepatitis, osteomyelitis, hemaglobinuria, and bighead diseases in animals.

Clostridium novyi is divided into four types designated as types A through D, based on the production of toxins and other biologically active substances; *C. botulinum* includes a very heterogeneous group of strains that are divided into types A through G, based on the antigenic specificity of the neurotoxins that are produced. The strains of these seven types can be separated into four groups according to their physiological and serological characteristics (*1–3*). The strains of *C. botulinum* types C and D form one of these groups and share some of the same characteristics possessed by *C. novyi* type A (*1, 2*). The main difference between the species is in the toxins produced.

It has been reported previously that the change from nontoxigenicity to toxigenicity in *C. botulinum* types C and D requires the active and continued participation of bacteriophages (*4–7*).

This report provides evidence that a nontoxigenic, phage-sensitive strain of *C. botulinum* type C can be converted to another toxigenic species, *C. novyi* type A, after infection by phage NA1 from *C. novyi* type A. In addition, this same nontoxigenic, phage-sensitive bacterial strain can be converted back to toxigenic *C. botulinum* type C after infection by phage 3C of *C. botulinum* type C or to toxigenic *C. botulinum* type D by infection with phage 1D of *C. botulinum* type D. Three immunologically distinct toxins can therefore be produced by a common bacterial strain following infection by specific bacteriophages. Furthermore, the persistence of the toxigenic characteristic and type of toxin produced requires the continued presence of these same bacteriophages.

Spores of toxigenic *C. botulinum* type C strain 162 were treated with heat at 70°C for 15 minutes, diluted, and plated on trypticase-yeast-glucose (TYG) agar (*8*). Isolated colonies were cultured in TYG broth and tested for toxigenicity by the mouse assay (*8*) and for sensitivity to the bacteriophages of the toxigenic parent culture by the agar-layer procedure (*8*). Of the 40 isolates tested, one strain designated as HS37 had simultaneously lost its prophage and ceased to produce toxin. Strain HS37 was subcultured about 20 times in fortified egg-meat medium (*9*) over a period of 1½ years, and it re-

Table 1. Infection of cured nontoxigenic bacterial strain HS37 with *Clostridium botulinum* type C phage 3C, type D phage 1D, and *Clostridium novyi* type A phages NAI and NA2 and their effect on toxigenicity.

Phage	Number of cultures			Toxin neutralized by antiserum of
	Toxigenic	Produced phage*	Tested	
$3C^{tox+}$	40	40	40	*C. botulinum* type C
$1D^{tox+}$	40	40	40	*C. botulinum* type D
$NA1^{tox+}$	40	40	40	*C. novyi* types A and B
$NA2^{tox-}$	0	40	40	

* Cultures were also resistant to infection by homologous phage.

mained nontoxigenic and sensitive to the bacteriophages of the toxigenic parent strain 162. As a further test for toxicity, cells from HS37 were disrupted by sonification and assayed for toxin, but the cultures remained nontoxic with or without treatment with trypsin.

Strain HS37 was tested for its sensitivity to bacteriophages of different strains of *C. botulinum* and *C. novyi* type A with the agar-layer procedure. In addition to being sensitive to the phages of the parent type C strain 162, strain HS37 was also sensitive to the bacteriophages of *C. botulinum* type D strain South African and of *C. novyi* type A strain 5771. Bacteriophage $3C^{tox+}$, which produces turbid plaques 0.5 to 3 mm in diameter on lawns of strain HS37, was isolated from type C strain 162. Bacteriophage $1D^{tox+}$ from type D strain South African also produced turbid plaques (1.0 to 2 mm in diameter) on strain HS37.

Strain HS37 was sensitive to two bacteriophages designated as $NA1^{tox+}$ and $NA2^{tox-}$ from *C. novyi* type A strain 5771. Phage NA1 produces turbid plaques (1.0 to 3.0 mm in diameter), whereas phage NA2 produces smaller turbid plaques (0.5 to 1.0 mm in diameter) on strain HS37. These plaques were purified by six successive single-plaque isolations on strain HS37 by procedures previously described (*8*). Bacteriophage stocks were produced by propagating the purified bacteriophage with strain HS37 in TYG broth at 33°C. Bacteriophage stocks were treated with 100 μg of deoxyribonuclease II (Sigma) per milliliter of phage solution for 2 hours at 37°C and then filtered to remove bacterial cells. Deoxyribonuclease was used to rule out the possibility of a transformation principle by deoxyribonucleic acid. All filtrates were tested for absence of bacterial cells by inoculating several milliliters of filtrate into TYG broth and fortified egg-meat medium and then incubating the cultures for several weeks at 33°C.

The relation of these four purified phages to the toxigenicity of strain HS37 was tested by plating dilutions of each filter-sterilized phage preparation with the recipient nontoxigenic culture HS37 according to the agar-overlay procedure. Material from the center of isolated plaques was transferred into TYG broth and incubated at 33°C for 5 days. These cultures were then assayed for toxin, tested for bacteriophage production with HS37 as the indicator strain, and tested for sensitivity to the homologous phage. The type of toxin produced was identified by the mouse protection test (*8*) with use of monospecific antiserums of *C. botulinum* types A through G and *C. novyi* types A and B.

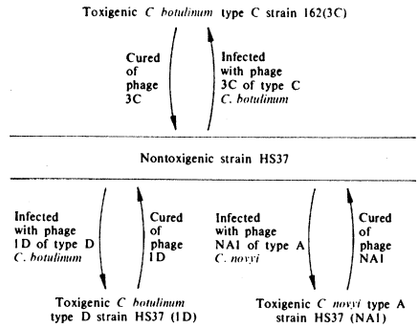

Fig. 1. The relation of bacteriophages 3C, 1D, and NA1 to the type of toxin produced by bacterial strain HS37.

Table 1 summarizes the relation of phages 3C, 1D, NA1, and NA2 to the toxigenicity of bacterial strain HS37. All broth cultures arising from picked plaques produced by these four different phages continued to carry the respective phage and, in turn, were immune to infection by the homologous phage. When HS37 was infected with phage 3C, toxin neutralized only by antiserum to *C. botulinum* type C was produced. Infection of strain HS37 with phage 1D also resulted in toxigenic cultures, but this toxin was neutralized by antiserum to *C. botulinum* type D. Phage NA2 of *C. novyi* had no effect on the toxigenicity of strain HS37, but phage NA1 did induce the production of the alpha toxin of *C. novyi*. The alpha toxin (which is produced by both types A and B of *C. novyi*) was neutralized by antiserum to both types A and B of *C. novyi*, but not by any of the antiserums to *C. botulinum*. The two main distinguishing characteristics between type A and B (*C. novyi*) are (i) type A grown on egg yolk agar produces an opalescent film on and around its colonies and (ii) type B produces the lethal, lecithinolytic beta toxin. Neither type A nor type B strains exhibit both of these characteristics (*2*). Nontoxigenic strain HS37 used in the current studies also produces this opalescent film on and around its colonies. When strain HS37 is infected with phage NA1 it continues to produce the opalescence on egg yolk agar and also produces the lethal alpha toxin in broth cultures, but it does not produce the beta toxin. In addition, culture HS37 infected with phage NA1 was confirmed by L. Ds. Smith from Virginia Polytechnic Institute as *C. novyi* type A. The cells of HS37 infected with phage NA1 also fluoresced brightly when stained with a fluorescein-labeled antiserum against *C. novyi*. Based upon these characteristics, strain HS37 infected with phage NA1 would be identified as *C. novyi* type A.

To determine whether the continued participation of the phages was required to maintain the toxigenic character of HS37, the toxigenic cultures carrying the phages were again cured of their prophages in the following manner: Strain HS37 infected with phage NA1 and producing *C. novyi* toxin was permitted to sporulate and the spores were treated with heat, as previously described, and plated on TYG agar. Isolated colonies were transferred into TYG broth and tested for toxicity, phage production, and phage immunity. Of the 40 isolates tested, 25 continued to produce *C. novyi* toxin and phage NA1 and, in turn, were immune to the infection by phage NA1.

Toxigenic isolates from this experiment were again selected, and sporulated cultures were treated with heat and plated on TYG agar. Isolates were grown in TYG broth and these cultures were tested for toxigenicity, phage production, and phage immunity. Of the 50 isolates tested from this experiment, 46 continued to produce the *C. novyi* toxin and phage NA1 and were resistant to infection by phage NA1. The nontoxigenic cultures from both experiments were sensitive to phage NA1 and also to phages 3C and 1D, and when infected with these phages they produced *C. novyi* and *C. botulinum* type C and type D toxins, respectively. These same experiments were used to establish the relation of phages 1D and 3C to the toxigenicity of strain HS37. In all cases, the toxigenicity and type of toxin were directly related to the continued participation of the specific bacteriophage.

Figure 1 summarizes these experiments. Strain HS37 is a nontoxigenic derivative of toxigenic *C. botulinum* type C strain 162. When cured of phage 3C it becomes nontoxigenic and sensitive to phages 3C, 1D, and NA1. Strain HS37 can then be infected with either of the three phages, and when infected it produces *C. botulinum* type C and type D and *C. novyi* alpha toxin, respectively. These cultures can then be cured of their phages and the cycle can be repeated again. When strain HS37 is infected with phage 3C or 1D, it is im-

mune to infection by both 3C and 1D phages, but it continues to be sensitive to infection by phage NA1. Conversely, strain HS37 infected with phage NA1 is immune to infection by the homologous phage, but remains sensitive to phages 3C and 1D.

Electron micrographs were prepared of the bacteriophages according to procedures of Eklund and Poysky (*8*); photomicrographs of phages 3C and 1D were presented in a previous paper (*8*). Phage NA1, which is responsible for inducing nontoxigenic *C. botulinum* type C to produce the alpha toxin of *C. novyi*, exhibits a polyhedral head 65 nm in diameter and a tail 160 nm long and 5 nm in diameter. The sheath surrounding the tail is 68 nm long and 20 nm wide. Phage NA1 of *C. novyi* is similar in morphology to the phages that induce toxicity of *C. botulinum* types C and D (*8*).

These studies show that *C. botulinum* type C strain 162 ceases to produce toxin when cured of its prophage. This nontoxigenic derivative can then be converted to another toxigenic species, *C. novyi* type A, by infection with bacteriophage NA1 or converted to *C. botulinum* type C by bacteriophage 3C or to *C. botulinum* type D by bacteriophage ID. The toxigenicity and the type of toxin produced by this common bacterial strain depends upon the continued participation of specific bacteriophages. Further studies are needed to determine whether *C. novyi* type A can be cured of its prophage and then converted to *C. botulinum* type C or type D by bacteriophages.

The finding that *C. botulinum* type C can be converted to *C. novyi* type A opens new areas of research in the formation of these different toxins and possible control of botulism and gas gangrene in man and animals. Our results suggest that in nature a common bacterial strain could be responsible for both diseases. The specific bacteriophage that infects this common bacterial strain would therefore govern not only the toxigenicity of the bacterium but also the resulting disease.

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Aversive Situational Effects on Alpha Feedback Training

Abstract. *Anticipation of electric shock did not depress alpha activity in a feedback situation though it was associated with reported anxiety and heightened arousal indexed by greatly increased heart rate and number of spontaneous skin conductance responses. Contrary to previous reports, a reduction in alpha activity is not a necessary consequence of apprehension or heightened arousal.*

Considerable public and professional interest has been aroused in the use of alpha feedback training. The many beneficial results that have been claimed for the technique appear to be based on observations that anxiety-arousing stimuli and situations that are associated with sudden increases in levels of arousal produce sharp, dramatic, transient drops in electroencephalographic (EEG) alpha density. As early as 1929, Berger noted that worry and apprehension result in depression of the alpha rhythm (*1*). After reviewing the literature, Williams (*2*) considered the depression of alpha activity with apprehension a well-established phenomenon. It has generally been assumed, therefore, that as individuals are trained to augment alpha density they concomitantly learn to regulate their level of arousal and also gain control over the degree of experienced anxiety.

We have previously noted that alpha feedback training does not allow individuals to exceed their own optimal baseline, obtained from a nondrowsy subject under relaxed conditions with eyes closed in a totally darkened room (*3*). Under conditions when alpha density had initially been depressed by ambient light, however, subjects could learn to produce alpha activity with their eyes open, a circumstance which had previously interfered with alpha production, by learning to ignore their surroundings and avoiding use of their oculomotor system. It seemed likely that if anxiety suppresses alpha activity, individuals learning to augment this rhythm with feedback training would simultaneously be learning to prevent the increased physiological arousal and the subjective components of the anxiety experience.

The present study sought to test this rationale empirically, and focused on the relationship between both baseline alpha density and alpha density in a feedback situation under conditions of experimentally varied anxiety and arousal levels. Male college students were solicited for a study in EEG conditioning, and were told that the study would involve one session of physiological recording. During this screening session, considerable care was taken to establish rapport with each subject as occipital (O^2) to right mastoid EEG, electrooculographic, electrocardiographic, and skin conductance electrodes were attached. Records were obtained in both darkness and in dim ambient light, including eyes-open and eyes-closed 3-minute baselines and three 5-minute trials under each light condition, using alpha identification and auditory feedback procedures described previously (*3, 4*). Only after the completion of this first session were those individuals who had at least 25 percent alpha density during the initial eyes-closed baselines asked whether they would be interested in returning for a further experiment involving harmless but quite painful electric shock. It was our intent to create an ambiguous situation where subjects would be moderately anxious for the succeeding session.

From the 22 subjects in the first session who were asked to participate