References and Notes

1. L. M. Roth and E. R. Willis, *Smithsonian Inst. Misc. Collections* **122**, 1 (1954).
2. R. H. Barth, Jr., "Comparative and experimental studies on mating behavior in cockroaches," thesis, Harvard University (1961).
3. These females, when tested for receptivity, were exposed to males in metal containers, 12 by 18 by 7 inches, that were covered to exclude most of the light. Males 10 or more days old, at which time they usually show courting behavior, were used (2♂ : 1♀). Although receptive females usually mate within a few minutes under these conditions, each test period lasted 1 hour.
4. A female mated when 6 days old would not mate again for about 60 days (approximately 10 days to oviposition following copulation plus about 50 days of gestation; these periods vary with temperature).
5. F. Engelmann, *Experientia* **16**, 69 (1960).
6. R. H. Barth, Jr., *Science* **133**, 1598 (1961); *Gen. Comp. Endocrinol.* **2**, 53 (1962).
7. L. M. Roth and E. R. Willis, *Am. Midland Naturalist* **47**, 66 (1952).
8. D. Bodenstein and I. B. Sprague, *J. Exptl. Zool.* **142**, 177 (1959).
9. Engelmann stated that females of *Leucophaea* mate only if the titer of corpus allatum hormone is low and once the hormone concentration increases the females do not mate (*5*). We have found, to the contrary, that virgin *Leucophaea* will mate after their oöcytes have grown markedly in size, which may be indicative of a high concentration of hormone (*11*).
10. The oöcytes of most *Nauphoeta* females do not contain yolk when parturition occurs, but in some individuals inhibition of the corpora allata apparently ceases prior to parturition and yolk is present in the oöcytes when the female gives birth (*11*).
11. L. M. Roth and B. Stay, *Psyche*, in press.
12. ———, *Science* **130**, 271 (1959); *J. Insect Physiol.* **7**, 186 (1961).
13. L. M. Roth and E. R. Willis, *J. Exptl. Zool.* **128**, 489 (1955).
14. One trial consisted of exposing females to males (2:1) for 1 hour once a day.
15. A. Manning, *Nature* **194**, 252 (1962).
16. During one of the trials ten females that had mated were again exposed to males immediately and nine of them mated again.
17. The abnormal insertion of the spermatophore into females with transected nerve cords has been reported for *Leucophaea* (*11*).
18. This study was made while I was a postdoctoral fellow at Harvard University.

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Aversive Training: Long-Term Effects

Abstract. Three years ago a tone ending in unavoidable electric shock was periodically presented to pigeons while they pecked a key for food. When pecking was disrupted by tone, shock was disconnected and the training tone and tones of different frequencies were presented. At first all tones caused a reduction in the rate of pecking, but as testing proceeded the gradient narrowed. In our study, testing was resumed after a 2½-year interruption. Despite the interruption the tones still suppress behavior.

The study reported here was concerned with the retention of a conditioned emotional response. Such a response results from a training history in which a neutral stimulus has typically preceded an unavoidable noxious event. It can be detected by determining whether subsequent presentations of the stimulus cause a reduction in the rate of ongoing hunger-motivated responses (*1*).

In earlier work (*2*) we investigated the degree to which this reduction (conditioned suppression) was mediated by stimuli which were like, but not identical to, the stimulus that was used in the original training. This phenomenon, the stimulus generalization of conditioned suppression, commanded special attention because it represents one of the mechanisms by which aversive training can affect large segments of an organism's behavior (*3*). In order to examine it, a 48-second tone accompanied for the last 8 seconds by unavoidable electric shock was periodically presented to pigeons (maintained at 80 percent of their normal body weight) while they pecked a key for food. When pecking was completely disrupted by the 40 seconds of preshock tone, the use of shock was discontinued and tests for stimulus generalization were begun. In these test sessions, the tone used in training (1000 cy/sec) and tones of different frequencies (300, 450, 670, 1500, 2250, and 3400 cy/sec) were presented sequentially and in random order, without shock, at intervals of approximately 10 minutes. Each tone lasted 40 seconds, and all tones were equated for intensity (80 db sound pressure level relative to 2×10^{-4} dyne/cm^2). The entire series of seven tones was presented once per session, and sessions occurred every day.

Initially, the gradient of generalization was broad: all tones caused some degree of conditioned suppression. As testing proceeded, however, the gradient systematically narrowed until only the tone used in training produced substantial reductions in the rate of pecking. These results were of particular interest for a least two reasons. (i) They appeared to reflect the development of a discrimination without either previous or concurrent differential reinforcement. (ii) The extinction of conditioned suppression appeared to proceed at a very slow pace (*3*). In our study we sought to examine these effects further. In particular, we wished to determine how the gradient changes with the passage of time.

We used two of the surviving birds (Nos. 21 and 9) from the earlier experiments. In that work testing had been discontinued when the birds exhibited a fairly strong tendency to suppress pecking in response to the tone that had previously been paired with shock and a reduced tendency to suppress pecking in response to the other test tones. At the termination of the tests the birds were returned to their home loft, where they were kept for approximately 2½ years. Then, with no further aversive training, testing was continued—that is, the seven tones were presented sequentially and in random order (without shock) while the birds pecked a key for food. The experimental situation in these tests was identical with that in the previous work with these birds.

Figure 1 shows the results of these recent tests (solid lines) as well as the results of the tests that had been conducted more than 2 years earlier (dashed lines). Each gradient represents data averaged across four successive sessions. The top gradient for bird No. 9 represents the first block of sessions for that bird. The top gradient for bird No. 21 represents the second block of sessions for that bird (*4*). The remaining gradients, for both birds, represent successive blocks of sessions, in descend-

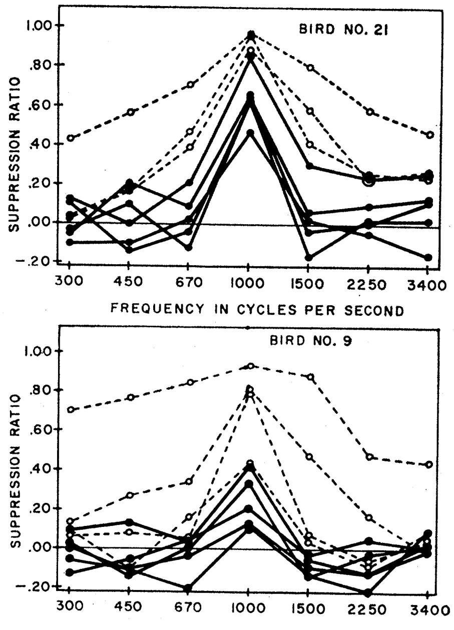

Fig. 1. Generalization gradients for birds Nos. 21 and 9. The suppression ratio equals (pre-tone R's − tone R's) / (pre-tone R's). The solid lines show the results of the sequence of tests reported here, whereas the dashed lines show results obtained more than 2 years previously. Each gradient represents data averaged across four successive sessions. The top gradient for bird No. 9 represents the first block of sessions for that bird. The top gradient for bird No. 21 represents the second block of sessions for that bird. The remaining gradients, for both birds, represent successive blocks of session, in descending order.

ing order. The ordinates show the mean suppression ratio during a given tone. This index compares the number of responses (tone R's) made in the 40-second tone period to the number of responses (pre-tone R's) made in the 40-second period prior to presentation of the tone.

Numerically, the relationship is expressed as a ratio:

(pre-tone R's — tone R's) / (pre-tone R's)

As can be seen in Fig. 1, during the earlier tests, generalization of suppression was broad at first, but as testing proceeded the gradients narrowed. In general, the interruption produced very little effect on the course of the gradients. For bird No. 21 the 1000 cy/sec tone exhibited no loss whatsoever, and the decrements for the other tones are no larger than would be expected had testing proceeded without interruption. The results of bird No. 9 are similar except that for this bird the tones on the wings of the gradient had ceased to cause suppression prior to the interruption.

An unexpected feature of the results is the finding that during the terminal stages of testing a disproportionately large number of negative ratios was obtained for tones on the wings of the gradient. For example, during the final eight sessions for bird No. 9 and during the final four sessions for bird No. 21 the several stimuli on the wings of the gradient yielded more than twice as many negative ratios as positive ratios. A *t*-test on the difference between the number of responses in the tone periods and in the pre-tone periods for these stimuli yielded $t = 2.156$ with 23 degrees of freedom for bird No. 21 and $t = 2.785$ with 47 degrees of freedom for bird No. 9. Since values of t as large as this would occur with $p < .05$ on the basis of random sampling, we concluded that the observed tendency for the ratios to dip below zero represents a reliable effect. Apparently a stage was reached during the final phases of the extinction procedures in which many of the tones tended to facilitate pecking. Unfortunately we do not yet understand the mechanisms which underlie this curious phenomenon.

The main features of these results, however, are consistent with results of several other studies which show that conditioned responses are retained over long periods (*5*). In this respect, our results provide a dramatic illustration of the proposition that the mere passage of time plays little, if any, role in the elimination of the negative effects of averse training (*6*).

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3. S. A. Mednick and J. L. Freidman, *Psychol. Bull.* **57** (1960).
4. The results of the first block of sessions for bird No. 21 are not shown because during these sessions the bird was maintained at 70 rather than 80 percent of its normal body weight. See H. S. Hoffman and M. Fleshler, *J. Exptl. Anal. Behavior* **4** (1961), for a discussion of the behavioral consequences of testing subjects at this weight level.
5. G. H. S. Razran, *J. Exptl. Psychol.* **24** (1939); G. R. Wendt, *J. Gen. Psychol.* **23** (1938).
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Homology Test between the Nucleic Acid of an RNA Virus and the DNA in the Host Cell

Abstract. Deoxyribonucleic acid in the host cell does not contain a sequence complementary to the nucleic acid of an RNA virus. Specific formation of hybrid between deoxyribonucleic acid and ribonucleic acid was used as a detecting device. The test was internally controlled and sensitive enough to reveal complementary stretches in the DNA corresponding to 10 percent of the viral ribonucleic acid. The implications of the results for replication and transcription of RNA are discussed.

In the last several years there has been an accumulation of data consistent with the concept that polyribonucleotides are the informed intermediaries between DNA-genomes and the cellular entities which carry out their instructions (*1, 2*). However, the problems posed by the existence of viruses which use RNA for the transmission and storage of genetic information remain for detailed resolution.

A definitive decision on the existence or nonexistence of homology between viral RNA and the DNA from its host would aid in delineating the mechanisms of replication of RNA and transcription from an RNA genome.

For technical convenience the system chosen was *Escherichia coli* (Hfr strains, K-16 and K-10) and an RNA bacteriophage related to the one (f2) discovered by Loeb and Zinder (*3*). The virus used, MSϕ2, (*4*) had biological, chemical, and physical properties similar to those reported for f2 (*5*).

A decision on whether or not there is homology between two nucleic acid molecules rests essentially on a comparison of base sequences. In practice what can be readily detected is sequence complementarity (*6, 7*). Experimentally, then, the question we seek to answer may be posed as follows: Does the DNA found in the host cell contain, either before or after infection, a sequence complementary to the nucleic acid of an RNA virus?

One answer to questions of complementarity is provided by specific hybrid formation. A sensitive test for hybridization of RNA and DNA was developed by Hall and Spiegelman (*8*) to demonstrate that the RNA synthesized in *E.* coli after infection by the bacteriophage T2 is complementary to the DNA of the virus rather than to that of the host. Equilibrium density centrifugation in swinging bucket rotors combined with isotopic labeling was used to identify the hybrid structures. The same procedures were successfully used to exhibit complementary RNA synthesized in cells during "step-down transitions" (*9*) or short pulses (*2*), as well as RNA formed in vitro by the DNA-dependent RNA polymerase (*10*).

The investigations just cited tested heterogeneous populations of RNA which represent between 10 and 100 percent of the available sequences in DNA. Consequently, the detection of hybrids did not strain the available sensitivity. However, the numerical situation inherent in the present problem is far less favorable. It demands finding one particular sequence in the DNA equivalent in length to the viral RNA. Since the molecular weight of the viral RNA is about 8×10^5, one must detect complexes including approximately 0.005 percent of the total length of *E. coli* DNA. However, a similar numerical difficulty was overcome by Yankofsky and Spiegelman (*11, 12*) when they established the existence of sequences in DNA complementary to sequences in homologous ribosomal RNA. These experiments