Reports

Deoxyribonuleic Acid Mediates Sensitization of Penicillin-Resistant Staphylococcus

Abstract. An extract, which contained deoxyribonucleic acid, from a penicillin-sensitive hospital strain of *Staphylococcus aureus* conferred the property of sensitivity upon a penicillin-resistant strain of the same species, with the phage type of the sensitized *S. aureus* remaining unchanged after transformation.

In 1949 George and Pandalai (*1*) demonstrated the sensitization of *Escherichia coli* to penicillin in the presence of a nucleic-acid fraction isolated from a sensitive strain of *Staphylococcus aureus*. Evidence of DNA-mediated transfer of penicillin sensitivity between two bacteria of the same species has been lacking. The present study (*2*) reports the sensitization of a penicillin-resistant strain of *S. aureus* in the presence of a DNA-containing extract of a sensitive strain of the same species.

The two strains of *S. aureus* chosen were isolated from hospital patients. The resistant receptor strain was phage type 73/80/81, a particularly resistant staphylococcus (*3*), and the sensitive donor strain was phage type 55.

The preparation of the sensitive staphylococcus transforming principle was carried out essentially by the method applied by Alexander and Leidy (*4*) to type c *Hemophilus influenzae*. The sensitive strain was grown for 8 hours in 40 erlenmeyer flasks, each containing 100 ml of trypticase soy broth. The yield was centrifuged, the supernatant was discarded, and 45 ml of a solution containing 0.1*M* sodium chloride and 0.1*M* sodium citrate was added to the cells. After freezing with a mixture of carbon dioxide ice and 95-percent ethyl alcohol, the cells were thawed at 4°C, and the volume was increased to 500 ml by the addition of citrate saline solution and 5 ml of 10-percent sodium deoxycholate. After the solution cleared, protein was removed by three extractions with chloroform and amyl alcohol. A fibrous precipitate was obtained by adding two volumes of absolute ethyl alcohol to the partially deproteinized solution and allowing it to stand for 12 hours at 4°C. This precipitate was dissolved in 0.85-percent sodium chloride at *p*H 7.0, deproteinized twice more as described above, and again precipitated with two volumes of absolute alcohol. For use in the experiment, the final fibrous precipitate was washed in absolute alchohol and dissolved in 30 ml of physiologic saline at *p*H 7.0.

Antiserum reactive with resistant *Staphylococcus aureus* was prepared by injecting guinea pigs intraperitoneally with a gradually increased inoculum of the resistant strain, which contained approximately 1.5 to 2.0 billion cells per milliliter in 0.2 percent formalinized saline. The initial dosage of 0.1 ml was increased by 0.1 ml daily for the first 4 days of each week, until a final dosage of 1.0 ml daily was attained. Seventeen days after the initial injection, the animals were bled, and the serum obtained was heated to 60°C for 30 minutes.

Tubes containing a 2-mm loopful of a 6-hour culture of the resistant strain of *S. aureus*, 0.5 ml of the antiserum, 2 ml of trypticase soy broth, and 0.1 ml of the transforming extract were incubated at 35°C for 16 hours. Additional tubes were prepared by incubating resistant *S. aureus* culture and antiserum for 2, 4, and 6 hours, then adding the broth and transforming extract, and incubating for an additional 16 hours. The control consisted of a 2-mm loopful of the resistant *S. aureus* strain and 2 ml of broth, incubated at 35°C for 16 hours. After 16 hours of incubation, the differently treated cultures and controls were spread uniformly on nutrient agar plates with a 10-unit, 7-mm penicillin disk in the center of each dish. The plates were incubated for 24 hours at 35°C.

Zones of inhibition within 3 mm of the 13-mm size (measured from edge of disk) of the zone of inhibition of the original sensitive strain were obtained on all plates except those of the controls, which showed no inhibition zone. No colonies grew in any of the zones of inhibition. Colonial growth in all the plates containing the transforming extract was moderate. The growth of the controls and the original sensitive strain was abundant.

The same procedure described above, modified only by the addition of deoxyribonuclease (*5*) in the tubes with the transforming extract, resulted in no zones of inhibition. Subtransfers were not made.

Attempts to sensitize penicillin-resistant *S. aureus* in vivo are suggested by these results.

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References and Notes

1. M. George and K. M. Pandalai, *Lancet* **I**, 955 (1949).
2. The work described in this report was done largely in my home laboratory. I am grateful to Earle Borman, director, and Dr. Walter Karakawa, research microbiologist, of the State of Connecticut Health Laboratories, for making available those facilities which I lacked at home.
3. J. E. Blair and M. J. Carr, *J. Am. Med. Assoc.* **166**, 1192 (1958).
4. H. E. Alexander and G. Leidy, *Proc. Soc. Exptl. Biol. Med.* **73**, 485 (1950).
5. Furnished through the kindness of Worthington Biochemical Corp., Freehold, N.J.

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Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

Environmental Familiarity and Feeding in a Planarian

Abstract. Planarians, in common with higher animals, tend to delay feeding in environmental conditions to which they are not habituated.

If a higher animal, for example, the rat, is placed in a strange situation, its feeding behavior is suppressed until it becomes familiar with the novel environment. This has the experimental consequence that animals fasted a given

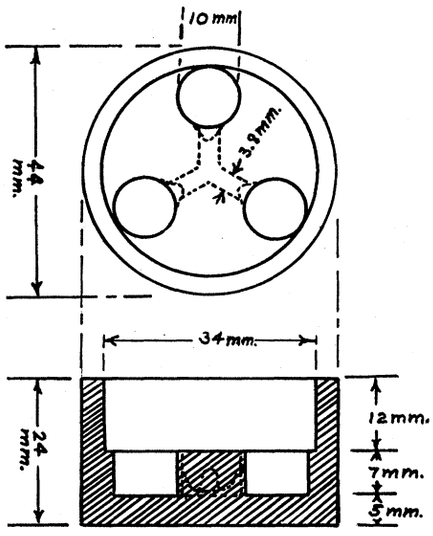

Fig. 1. Top and side views of the Lucite receptacle that was used as the unfamiliar test environment for planarians.

length of time will delay feeding longer when placed with food in a strange environment than they will when placed in a familiar one. This response, characteristic of at least a number of species of mammals, is generally conceded to be related to some variety of anxiety state in the animal (*1*). More interesting is that this differential latency provides an operational method of ascertaining the degree of strangeness or familiarity perceived in an environmental setting by any animal exhibiting such an effect. Distinctions of this type are apparently accomplished via two different mechanisms, both of which play a role in higher animals such as the rat (*2*). The animal may leave some marker spoor which it detects on revisiting the environment. Or, it may distinguish certain patterns of cues characterizing the environment. The latter would seem to be closely akin to a crude variety of cognitive memory, since without markers or memory all environments must be alien to an organism.

Planarians have recently been shown to have the capacity for classical (*3*) and instrumental conditioning (*4*). Because of its theoretical implications, the following experiment was undertaken to ascertain whether planarians exhibit the delayed feeding effect under circumstances in which the role of a marker spoor could be ruled out.

Planarians of the species *Cura foremani* were fed to satiation on raw liver and then fasted varying lengths of time in glass bowls 4 inches in diameter, containing about an inch of spring water.

Individuals of the group denoted as "unfamiliarized" were transferred into a small Lucite receptacle (see Fig. 1) into which two small pieces of raw liver and water from the home bowl of the planarians had been placed just previously. The peculiar shape of the Lucite receptacle derives from the fact that it was designed for some maze-learning experiments (*4*). It was used in the present study so the results could be employed to elaborate an unexpected effect of the maze study. The time was measured from the moment of introduction of the planarian into the test chamber until the planarian attached itself to one of the pieces of liver and started feeding. A different planarian was used for each such measurement.

Individuals of the group denoted as "familiarized" were placed in the Lucite receptacle used as described above which was filled with water from their home bowl, but without any liver, and allowed to remain there for a period of approximately 90 minutes. At the end of this period of time they were removed from the Lucite receptacle and placed back in their home glass bowl for about 25 minutes, during which time the Lucite receptacle was emptied and washed with hot water. These planarians were then tested in the Lucite receptacle with liver in the same manner employed for the "unfamiliarized" group.

To control for diurnal cycles (*5*) in their behavior, all tests were conducted during the hours between noon and 8:00 P.M. with familiarized and unfamiliarized individuals being tested in alternating order. On half of the days series of tests were started with a test on a "familiarized" individual; on half, with an "unfamiliarized."

Results are shown in Fig. 2. Each point in Fig. 2 depicts the mean of three measurements. A test of the difference in latencies between familiarized and unfamiliarized gives $t = 2.7$. Since there are 24 measurements and 8 different treatment groups, there are 16 residual degrees of freedom, which gives $P <$ .01 on a test that was single tailed (since the direction was predicted in advance).

Hence, two aspects of Fig. 2 are noteworthy. The familiarized group gives a significantly shorter latency for a given fast length than the unfamiliarized group. Second, the latency until onset of feeding as a function of fast length is similar to that observed in higher animals—that is, it decreases with length of fast to some minimum, then increases again slightly for very long fast times, except that the time scale is longer.

The alternating test sequence results in a latency test on an unfamiliarized planarian following each test on a familiarized one, in the same test chamber used throughout. Suppose the shorter latency of the familiarized planarian stems from incomplete removal of the spoor by the washing. One would be forced to conclude that every planarian distinguishes its own spoor from that of every other, inasmuch as the unfamiliarized planarian would also be tested in the presence of the same amount of residual spoor from the previous occupant of the chamber.

Planarians of both groups continue moving in the test chamber during their latency period in the food test, encountering the pieces of liver repeatedly during this period. Hence, the differential latency cannot be ascribed to a difference in activity that causes one group to find the food sooner.

Thus planarians exhibit the same dependency of the feeding latency upon

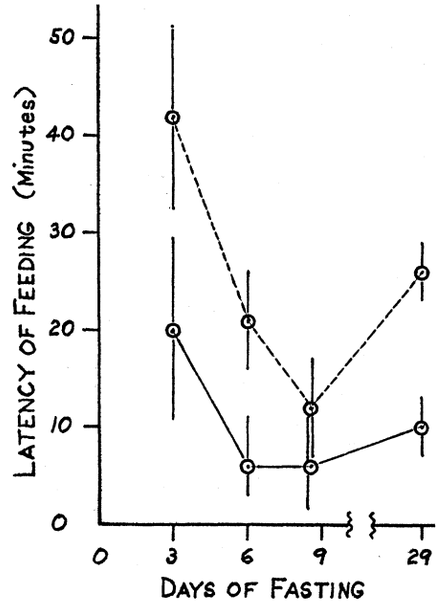

Fig. 2. Delay of feeding of *Cura foremani* in a test environment as a function of the number of days of previous fasting and in which there was (top) no previous exposure to environment, (bottom) previous 90-minute familiarization period in the environment. Vertical bars represent standard deviation of the mean of each measurement.

length of fast, and the delayed feeding effect, found in the higher vertebrates. Because the planarians are very far removed phylogenetically from the vertebrates and are among the most primitive animals that possess a central nervous system, bilateral symmetry, and encephalization, these, as well as other (*4*), behavior patterns may predate, and be more universal than, the vertebrate brain.

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Relationship between Locomotory Habits and Enzyme Concentration in Insects

Abstract. The activity of alpha-glycerol phosphate dehydrogenase has been measured in muscles from four insect species. In flying insects the enzyme activity is much greater per unit weight for flight muscles than for leg muscles. The results indicate a direct relationship between muscle levels of the enzyme and habitual mode of locomotion.

In contrast to most vertebrate smooth muscles, insect muscles possess low concentrations of lactate dehydrogenase and relatively large amounts of alpha-glycerol phosphate dehydrogenase (*1*). These enzyme differences are known to reflect differences in glycolytic pathways that are thought to be related to the very different efficiencies of vertebrate and insect muscles. As there are very large differences between insect species in habitual modes of locomotion, we have conducted experiments to determine whether these differences can be related to enzyme activities in insect muscles.

Four insect species were examined. The bumble bee *(Bombus terrestris)* is a strong flier and uses this means for its collection of food. The praying mantis *(Orthodera ministralis)* does fly but also often walks. The katydid *(Caedicia simplex)*, although winged, rarely flies and depends on walking as its almost sole means of locomotion. The tree weta *(Hemideina thoracica)* is wingless.

Adult specimens of these four species were captured locally. Flight muscles were dissected from the winged insects, and thoracic muscles, corresponding to flight muscles, from the wetas. Leg muscles were removed from all four species. All muscles were dissected into ice-cold, 0.9-percent potassium chloride solution and their weight was derived by difference.

The muscles were ground in a Potter-Elvehjem, all-glass, tissue homogenizer with more of the cold KCl solution. The resulting homogenate was centrifuged at 13,000 rev/min for 20 minutes at 2°C. The supernatant was used as the source of alpha-glycerol phosphate dehydrogenase.

Activity of the enzyme was determined by the following method, which is adapted from that of Chefurka (*2*). A mixture was prepared containing 0.37 μmole of dihydroxyacetone phosphate (DHAP), 3.0 μmole of reduced nicotinamide-adenine-dinucleotide ($NADH_2$), enzyme extract, and 0.1*M* phosphate buffer, *p*H 7.4. The final volume was 3.0 ml.

The reaction was initiated by the addition of DHAP, and the enzyme activity was determined by following the oxidation of $NADH_2$ spectrophotometrically at 340 mμ at 25°C. A control was used containing no DHAP. A unit of alpha-glycerol phosphate dehydrogenase activity is defined as the amount producing an initial rate of oxidation of 0.01 μmole of $NADH_2$ per minute under the above conditions of assay. Results are given in Table 1.

It is apparent that flight muscles contain much more of the enzyme than leg muscles. Moreover, the activity of the enzyme in the flight muscles of the winged species seems directly proportional to the flying habits. There is a similar decrease in the alpha-glycerol phosphate dehydrogenase activity in the leg muscles. The flightless weta, however, has a moderate alpha-glycerol phosphate dehydrogenase activity in its leg muscles.

Table 1. α-Glycerol phosphate dehydrogenase in insect muscles.

Insect	Muscle	Enzyme (units per gram fresh wt.)
Bumble bee	Flight	4500
Bumble bee	Leg	2200
Praying mantis	Flight	1090
Praying mantis	Leg	175
Katydid	Flight	855
Katydid	Leg	85
Weta	Thoracic	250
Weta	Leg	475

These experiments appear to demonstrate a close relationship between the habitual locomotory habits of an insect and the alpha-glycerol phosphate dehydrogenase activity of its muscles (*3*).

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References and Notes

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2. W. Chefurka, *Biochim. et Biophys. Acta* **28**, 660 (1958).
3. This work is based upon a paper read to the New Zealand Institute of Chemistry Conference, August 1961. The work was supported in part by a grant to one of us (G.B.K.) from the Wellington Medical Research Foundation.

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Imprinting: Its Effect on the Response to Stress in Chicks

Abstract. Young chicks imprinted to surrogate mothers were compared with nonimprinted controls on two tests designed to measure resistance to stress. Half of each group was run with and half without a surrogate present during the stress. One test involving survival time under starvation showed no effects. However, in the other test, imprinted chicks showed fewer distress calls in response to auditory stimulation than nonimprinted controls.

Several writers have suggested a close relation between emotion and imprinting. Thus, maturation of fear may determine the critical period during which imprinting occurs (*1*). Conversely, imprinting may have as a main function the reduction of fear in the young animal (*2*). Gray (*3*) has reviewed evidence at the human level and suggests that, here also, lack of attachment to a parent or parent-surrogate has deleterious effects on emotional development.

The experiments reported here were aimed at providing further evidence on the relation between imprinting and emotion. Specifically, our aim was to examine both the effects of the im-