Table 1. Growth of compact- and diffuse-colony variants of *Staphylococcus aureus* (Smith) and colonies of *Staphylococcus aureus* (193) in normal mouse serum and in the mouse peritoneum.

Time (hr)	Growth of Smith strain of *S. aureus* in normal mouse serum		Growth of Smith strain of *S. aureus* in mouse peritoneum		Growth of *S. aureus* (193) in mouse peritoneum
	Viable diffuse cells/ml of serum	Viable compact cells/ml of serum	Viable diffuse cells/ml of exudate	Viable compact cells/ml of exudate	Viable (all compact) cells/ml of exudate
0	8.3×10^{1}*	5.2×10^{1}†	4.2×10^{5}	1×10^{6}	2.4×10^{6}
4			4.6×10^{6}	2×10^{6}	3.3×10^{6}
8			$> 2 \times 10^{9}$	1.3×10^{6}	8.6×10^{6}
10			$> 2 \times 10^{9}$	2×10^{4}	6.6×10^{5}
24	1.4×10^{7}*	1×10^{7}†	All mice dead (at 12 hrs)	$< 1 \times 10^{3}$ (All mice survived)	$< 1 \times 10^{3}$ (All mice survived)

* All diffuse colonies in plasma soft agar. † All compact colonies in plasma soft agar.

toneal washings from such mice, suitably diluted in plasma soft agar, have always shown all colonies to be diffuse, and indicate an in vivo selection of the few diffuse cells present in large volumes of older compact-type cultures.

Both the diffuse-colony and the compact-colony isolates of the Smith strain grew equally well, as indicated by plate counts, when incubated in normal human or mouse serum over a 24-hour period, but they developed at very different rates within the leukocytes in the mouse peritoneum. One was able to observe the progress of infections due to challenges of diffuse and compact isolates when peritoneal exudates of mice injected with the Smith strain were periodically sampled and stained with Wright stain. In the mouse peritoneum there was a prompt leukocytosis and a prompt phagocytosis of staphylococci, whether the challenge was of compact- or diffuse-colony origin. The phagocytized organisms from compact-colony isolates were viable, but there was little growth, as judged by plate counts and by estimation from stained-slide preparations.

What we observed with the diffuse-colony infection was quite different. There was a marked and consistent proliferation of the diffuse-colony staphylococci within the leukocytes for 8 to 12 hours, depending upon the challenge dose. During the interval between the eighth and twelfth hour, in the mice receiving the diffuse-colony challenge, there was an abrupt appearance of overwhelming numbers of extracellular staphylococci, and the mice died within the next 20 to 40 minutes. When a similar "shower" of extracellular organisms was observed in mice receiving large challenge doses of compact cultures of the Smith strain, these mice died, and examination of their peritoneal exudates showed that the organisms were all diffuse cells. Other strains of coagulase-positive staphylococci tested, developed as compact colonies in plasma soft agar, grew well in normal serum but, like the compact Smith strain, did not show significant growth in the leukocytes in the mouse peritoneum, although they remained viable for many hours. These relationships are shown in Table 1.

Rogers and Tompsett (*5*) have indicated that the disappearance of white blood cells from in vitro staphylococcus-leukocyte suspensions and the appearance of extracellular staphylococci might be due to the production of the leukocidin which Valentine (*6*) described as being lytic for the leukocytes. Jackson and Little (*7*) and Gladstone and van Heyningen (*8*) believe that this lytic leukocidin is delta-hemolysin.

Both variants of the Smith strain produced delta-hemolysin. Both were phagocytized by the leukocytes, and both remained viable for 12 hours in the white blood cells. The outstanding difference between the two isolates was their serological dissimilarity and the ability of the diffuse-type organism to develop readily within the leukocytes. Intracellular growth and production of leukolytic concentrations of delta-hemolysin without interference of inhibitory agents within the mouse leukocytes could then account for the sudden appearance of diffuse-type extracellular staphylococci. These organisms showed no evidence of clumping in the peritoneal exudate but disseminated freely. Sudden release of the organisms into the peritoneum, together with any toxic products formed by the staphylococci or lysed leukocytes, would account for the subsequently fatal outcome of the infection. Since the growth of the compact variant was inhibited within the white cells in the mouse peritoneum, there might not be a lytic concentration of delta-hemolysin produced to destroy the white cells. Dissemination of staphylococci and their toxic products hence would not take place.

Death of mice following intraperitoneal injection of very large challenge doses of other strains of coagulase-positive staphylococci suggests that the inhibitory activity within the phagocytes may be overcome by large numbers of cocci, or that incubation of the large number of viable phagocytized staphylococci may produce enough of the leukolytic agent to lyse the white cells and release lethal products to produce the delayed toxic death observed. The peritoneal washings of mice dying from large challenge doses of these strains show no diffuse colonies in plasma soft agar.

Futher study of the influence of delta-hemolysin and of specific antibodies upon staphyloccus infection is in progress. The study of rare staphylococcus mutants not ordinarily encountered by the general population of experimental animals should help to elucidate the role of coagulase and of inhibiting agents in infection.

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References and Notes

1. Cultures of the Smith strain of *Staphylococcus aureus* were received from Dr. William Steenken, Jr., Trudeau Laboratory, and from Dr. W. F. Verwey and Dr. K. Miller, Sharp and Dohme Division of Merck Institute for Therapeutic Research. The Smith strain is designated as S.A. 235 by some laboratories.
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Fractional Escape and Avoidance on a Titration Schedule

Abstract. Rats were shocked, continuously or intermittently, by an electrical stimulus whose intensity increased by one step every 20 seconds. Each time the rat depressed a lever in the experimental chamber, shock intensity was decreased by one step. Lever-pressing was maintained on such a program, with both continuous and intermittent delivery of shock.

Operant conditioning techniques can be used to acquire information about thresholds, or about the intensity or amount of a stimulus or reinforcer that will be tolerated or preferred. Here we report results obtained by a technique

that permits one to gauge the tolerance of a subject to electric shock (*1*). It is related to those used by Békésy, Blough, and Lindsley to measure, respectively, auditory thresholds, visual thresholds, and depth of sleep (*2*).

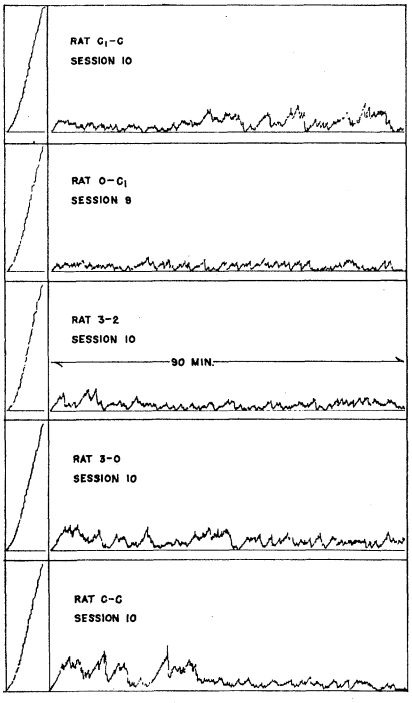

Fig. 1. Records for five animals on the fractional-escape schedule. The inset to the left of each record indicates the programmed rate of increase—that is, the rate at which the shock level would rise if the rat did not respond.

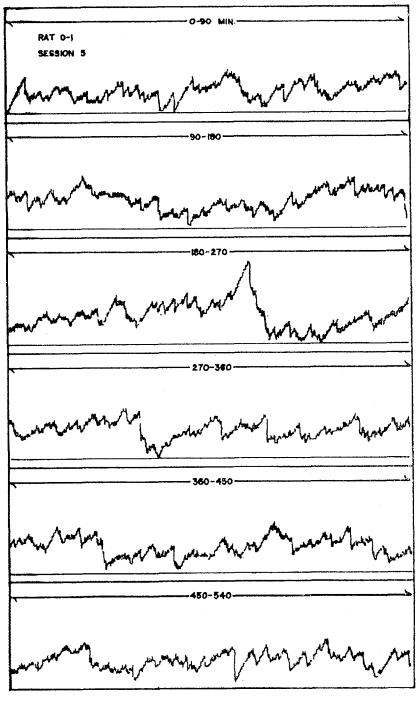

Fig. 2. Complete record of a 9-hour session on the fractional-avoidance program.

The technique employs a constant current shock stimulator whose output can be increased or decreased in discrete steps (*3*). The 30 steps between the minimum and maximum values of the output current are selected by a two-way stepping relay.

A pulse emitted by a timer activates the "increase" relay so that an increment in the shock level occurs every t seconds. For the data reported here, t was 20. The shock current is delivered to a floor of stainless steel rods in the experimental chamber, which is enclosed in a sound-deadened box (*4*) in a room adjacent to the one that contains the programming and recording apparatus. Each time the rat presses a lever in this chamber, a pulse is delivered to the "decrease" relay, and the stimulator output is reduced by one step. From such a program, which we have labeled a "titration schedule," we get a continuous assay of the rat's tolerance to electric shock. A recording millivoltmeter indicates the output of the stimulator.

A half-wave 60-cycle signal is the form in which the shock is delivered, with the wave form approximating a square wave. Two ranges were employed. The higher varied from 0.07 to 0.72 ma; the lower varied from 0.06 to 0.41 ma (*5*).

The titration method has been used in two different ways. In one, which might be called fractional escape, shock was applied continuously to the grid. Lever-presses reduced the intensity of this shock. The lower shock range was always used. In the other method of programming, which might be called fractional avoidance, a brief shock was delivered every 20 seconds, with each succeeding shock a step higher than the last. If the rat pressed the lever between shocks, the next shock he received was less intense by a number of steps equal to the number of responses he had made. Thus, responses did not avert but merely reduced the strength of the forthcoming shock. The lower range was used with three rats, the higher with two.

Five adult Wistar strain male albino rats have been studied with each method. All had been given preliminary experience on an avoidance schedule in which each lever-press postponed a brief shock (*6*).

Sample records from each of the five animals trained on the fractional-escape schedule are shown in Fig. 1. These represent the ninth or tenth 90-minute sessions for these subjects. The task was learned very quickly by the rats we used, and variability rapidly decreased with experience. At the stage of training shown in the figure, the amount of current tolerated by the rats did not shift very much within a session, nor did it vary appreciably from session to session. The distribution of responses in the 20 seconds between pulses to the "increase" relay was recorded in 2-second intervals. No single interval showed a preponderance of responses.

Each session on the fractional-avoidance schedule lasted for 9 hours. A representative record for a complete session is shown in Fig. 2. As in the fractional-escape procedure, no within-session or session-to-session trends in tolerance level could be discerned. The distribution of responses between shocks was recorded in 2-second intervals. Responses were most frequent in the first interval after the shock and dropped sharply afterward. Session-to-session changes consisted of slight decreases in the number of responses in this first interval. Since all five rats gave very similar response distributions, the mean percentage of responses falling in each interval was computed for the fifth session with the data from all five animals combined. These percentages were as follows: 33, 17, 10, 7, 6, 6, 6, 5, 5, 5.

Behavior on the fractional-escape schedule demonstrates that lever-pressing is maintained by fractional reductions in the intensity of a continuously applied noxious stimulus. Behavior on the fractional-avoidance schedule demonstrates that lever-pressing is maintained when the consequence of a response is a reduction in the intensity of a forthcoming shock.

The reinforcement for continuing to perform on the fractional-escape schedule is fairly clear, since each response produces an immediate decrease in shock intensity. What precisely motivates responding on the fractional-avoidance schedule is more obscure, although the response distribution indicates that most of the responses are elicited by the shock itself or by some state which the shock induces.

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1. This work was supported by grants from the United States Public Health Service [B-865 (C2)] and from the Institute for the Study of Analgesic and Sedative Drugs.
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3. The stimulator was designed by G. N. Webb, biophysics division, department of medicine, Johns Hopkins University School of Medicine. A report is now in preparation.
4. The sound-deadened box used in this study was manufactured by Foringer and Co., Rockville, Md.
5. Measured by a Hewlett-Packard 400 AB vacuum tube Voltmeter across a 100 kohm load. This instrument measures the average value of the wave. Because of the characteristics of the vacuum tube used in the stimulator, the first few steps are smaller than the others.
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