system as judged by gross observations of the animal behavior, even in large doses.

In the second category are *o*-nitro-, *p*-methoxy-, and the unsubstituted phenylboronic acid. These three compounds had a localization factor of nearly 1, but in most cases the brain had a slightly higher boron content. These compounds produced an immediate depressant action upon the animal's spontaneous activity and responsiveness to stimuli, and soon the animals were lying flaccid and supine, unresponsive to surgical operations. The aqueous-benzene partition coefficient was from 5 to 7.

In the final category are two compounds, *p*-tolylboronic acid and *p*-chlorophenylboronic acid. At the standard dose of 35 μg per gram of mouse, the compounds were highly toxic, and the LD_{50} was approached. Coma in these animals was often accompanied by generalized twitching of the limbs. Initially, both showed a tumor-to-brain localization of 0.2 to 0.3, and thus their behavior suggests that they encounter, not a barrier slowing their penetration into brain, but an avenue facilitating it. The water-benzene partition coefficient was from 1 to 2. It is apparent that these compounds which show maximal effects on the central nervous system and greater concentration in the normal brain relative to tumor do concentrate to a greater extent in the lipid solvent, benzene, whereas those which show no obvious effect on the central nervous system and low concentration in the brain have a much higher partition coefficient in an aqueous rather than in a lipid phase.

Initially, *p*-tolylboronic acid showed a localization factor 0.3, but gradually this ratio was reversed, and after 3 hours the tumor concentration was nearly twice the brain concentration. Methyl groups attached to an aromatic nucleus are readily oxidized in vivo to a carboxyl group—for example, toluene is transformed on ingestion to benzoic acid (*8*). If this type of conversion occurs with *p*-tolylboronic acid, then *p*-carboxyphenylboronic acid would be formed and this reversal would be understandable. *p*-Chlorophenylboronic acid, on the other hand, maintained a localization factor of 0.6 even after 3 hours.

An exception to this three-category division would appear to be *m*-aminophenylboronic acid. This compound shows an effect on the central nervous system only at doses of 70 μg per gram of mouse and yet the boron localization factor is nearly 1 and the aqueous-benzene partition coefficient is greater than 60. It is conceivable that this compound might be intermediate between groups 1 and 2 or possibly that a principle other than lipid solubility is involved.

In summary, it can be stated that increased solubility in a lipid solvent is an important measure of the penetration of the brain by a drug. Of the compounds which were examined, introduction of a methyl or a chloro substituent into an aromatic nucleus definitely enhanced the penetration of a molecule into the brain, while a carboxyl or carbamido substituent markedly inhibited its entrance.

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Acute Infection of Mice with Smith Strain of Staphylococcus aureus

Abstract. Two serologically distinct variants found in a unique strain of staphylococcus produce coagulase and are phagocytized, but only one is virulent to mice. Only virulent cocci grow rapidly within leukocytes. Leukocyte destruction by the virulent strain and release of many phagocytized cocci precedes mouse death. The leukocidic agent may be delta-hemolysin.

The Smith strain of *Staphylococcus aureus* (*1*), which was isolated in 1930 by Dubos and briefly described by Smith and Dubos (*2*), is an unusual organism. We have studied in detail the mouse infections produced by this strain because it is unique. An examination of the strain showed that there were at least two cellular types in the broth culture. This differentiation was most readily made when the plasma soft agar reaction described by Finkelstein and Sulkin (*3*) demonstrated the presence of both diffuse and compact colonies in the culture. Although both types of Smith colonies produce coagulase, as determined by the tube test, only the diffuse colony in the plasma soft agar was virulent to mice. We have encountered no other staphylococcus strain with such capacity for inducing an acute infection in mice when injected by the intraperitoneal route. We have found no other strain of coagulase-positive staphylococcus which produced diffuse colonies in soft agar containing normal plasma or serum.

Smith and Dubos (*2*) stated that the strain produced pigment, was coagulase-positive, and was phage type 44A/42E. We have observed that the strain readily produced delta-hemolysin. Very rarely one may observe a colony producing beta-hemotoxin, but most colonies did not produce demonstrable alpha- or beta-hemotoxins on sheep washed-blood-cell agar plates after incubation in 10 percent carbon dioxide. We have never observed the production of staphylokinase or bacterial protease by the organism, as judged by lysis of fibrin formed around colonies on fibrinogen agar plates. The intraperitoneal median lethal dose (LD_{50}) of the diffuse-colony culture in Swiss albino mice was approximately 4×10^6 viable cells per mouse when injected as a broth suspension, but, with 0.5 ml of 5 percent hog gastric mucin, the LD_{50} was about 580 cells per mouse. Other strains of *Staphylococcus aureus* isolated from lesions of human beings and of laboratory animals showed an intraperitoneal LD_{50} of 1×10^6 viable cells in mucin, but did not consistently cause death when 1×10^9 viable cells were injected without mucin.

The presence of diffuse and compact coagulase-positive colonies in the Smith strain cultures was confirmed when the strain was sent to Finkelstein and Sulkin (*4*). The latter described their observations and noted that the Smith compact isolate was agglutinated with absorbed Group II (Cowan) antiserum and was lysed by phage type 44A. The diffuse-colony isolate, however, did not agglutinate with Groups I, II, or III absorbed antisera, nor was it lysed by type 44A phage. This suggested that the two cellular types were serologically distinct and that the compact type contained an antigen not found in the diffuse cell.

We had observed that the separation of diffuse and compact colonies of the Smith strain by the plasma soft agar also separated the mouse-virulent colonies from the mouse-avirulent. Peritoneal washings from any mouse dying from infection by the Smith strain showed only diffuse-type colonies in plasma soft agar. Each colony type, when isolated and grown through four or five broth-to-broth transfers without reisolation, showed the presence of a few cells of the other type colony. Without reisolation, a compact-colony broth culture might contain a few diffuse-type cells. Large challenge doses of the compact-type cultures produced death in an occasional mouse, but peri-

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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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