4. Freedman, L. R., Beeson, P. B., *Yale J. Biol. and Med.*, 1958, v30, 406.
5. Rocha, H., Guze, L. B., Beeson, P. B., *ibid.*, 1959, v32, 120.
6. Yamauchi, H., Rosenau, W., Brainerd, H., Hopper, J., Jr., *Clin. Res.*, 1961, v9, 107.
7. Woods, J. W., *J. Clin. Invest.*, 1958, v37, 1686.
8. Shapiro, A. P., Kobernick, J. L., *Circ. Res.*, 1961, v9, 869.
9. Shapiro, A. P., *J. Lab. Clin. Med.*, 1960, v55, 715.
10. Jones, R. K., Shapiro, A. P., *J. Clin. Invest.*, 1963, v42, 179.
11. Beeson, P. B., Rocha, H., Guze, L. B., *Trans. Assn. Am. Phys.*, 1957, v70, 120.

Received July 17, 1963. P.S.E.B.M., 1964, v115.

Increased Susceptibility of Burned Rats to *Pseudomonas aeruginosa.** (28906)

RONALD J. MCRIPLEY AND DELNA W. GARRISON (Introduced by W. J. Nungester)
Department of Microbiology, University of Michigan, Ann Arbor

The problem of bacterial infection following extensive, severe burns has been the subject of intensive investigation. In addition to clinical studies this problem has been investigated utilizing laboratory animals. Rosenthal and co-workers(1-3) have long studied the role of infection in extensively burned mice. They have found that septicemia is an important factor in the delayed deaths of burned mice and that certain chemotherapeutic agents are effective in promoting survival. Goshi *et al*(4) reported that thermal injury in rabbits increased their susceptibility to *Staphylococcus aureus* and Liedberg(5) has shown that moderately burned guinea pigs also demonstrate increased susceptibility to *Pseudomonas aeruginosa*. The purpose of this study was to determine whether there was an increased susceptibility in burned rats to *Ps. aeruginosa* and if so, how soon this decrease in innate host resistance occurred following burns.

Materials and methods. Culture. A chloramphenicol resistant, streptomycin sensitive strain of *Ps. aeruginosa,* which had been isolated from a hospital patient, was used for all experiments. This strain was maintained on trypticase soy agar (BBL) and frequently passed through rats.

Burning techniques. Male albino rats of the Sprague-Dawley strain weighing 180 to 190 g were shaved. Burning was accomplished by means of a steam chamber which consisted of a metal cylinder with one side molded in the general shape of the back of the rat and connected at the other side to a steam generator. A rat anesthetized with sodium pentobarbital was placed on the chamber in an inverted position and burned for 30 sec. Full thickness burns of 30-34% of the total body surface were produced. To obtain 80-90% survival over the first 48-hour post-burn period, 0.85% NaCl solution equivalent to 15% of the body weight of a rat was injected intraperitoneally.

LD_{50} determination. The test organism after being passed through a rat was grown on trypticase soy agar for 20 hours at 37°C. Cells were harvested, washed 3 times with 0.1 M phosphate buffer (pH 7.0), and adjusted to a concentration of 3.4×10^9 cells/ml by means of a Klett-Summerson colorimeter (650 mμ filter). The number of organisms in the stock suspension was verified by replicate plates. Serial 10-fold dilutions were made and groups of 5 rats injected intraperitoneally, or subcutaneously with 0.1 or 1.0 ml aliquot of each dilution and observed for 28 days. Liver, spleen, and blood were cultured from all animals which died, and data were included only for those animals from which *Ps. aeruginosa* was recovered. The method of Reed and Muench(3) was used for calculating LD_{50}.

Results. In studying bacterial infection in burned animals it is necessary to distinguish

* This research was supported by USPHS Training Grant.

TABLE I. Effect of Burns on Susceptibility of Rats to *Ps. aeruginosa.*

Route of inj	Burned rats: Time of challenge (post-burn)	Burned rats: LD_{50}	Normal rats: LD_{50}
Subcutaneous (normal area)	2 min	<10 (100)*	2.0×10^9 (45)
	1 day	1.5×10^7 (60)	
Subcutaneous (burned area)	2 min	<10 (200)	
	1 day	<10 (200)	
	5 days	1.3×10^7 (60)	
	10 "	2.0×10^8 (50)	
	15 "	1.5×10^9 (40)	
Intraperitoneal	2 min	<10 (100)	
	1 day	1.9×10^5 (90)	5.6×10^7 (60)
	3 days	3.0×10^7 (60)	

* Figures in parentheses indicate No. of rats used for determination of LD_{50}.

between deaths due to the trauma of the burn and those resulting from infection with the test organism. Rats burned by the method described were generally resistant to the burn *per se* if fluid therapy was given since 90-96% of the rats survived the first 24 hours and 80-90% were living on the 28th post-burn day. In contrast, between 50 and 60% of the rats receiving no fluid therapy died within the first 24 hours. Burning produced a dramatic change in the susceptibility of rats to *Ps. aeruginosa* (Table I). Rats injected 2 minutes after burning, intraperitoneally or subcutaneously in the burned or non-burned area, were extremely susceptible to *Ps. aeruginosa* since an LD_{50} of less than 10 organisms was obtained in each case. Conversely, the LD_{50} for non-burned rats was extremely high; 2.0×10^9 organisms for the subcutaneous route of injection and 5.6×10^7 organisms for the intraperitoneal route, which demonstrates the innate resistance of the rat to *Ps. aeruginosa.* When burned rats were injected subcutaneously in the burned area, the marked susceptibility was still present 24 hours after burning, but resistance to the organisms gradually returned to normal as indicated by the LD_{50} of 2.0×10^8 bacteria on the 10th post-burn day and 1.5×10^9 bacteria on the 15th post-burn day. In the group of burned rats injected subcutaneously in a non-burned area, the LD_{50} 24 hours after burning was 1.7×10^7 organisms or 10^6 times greater than the LD_{50} for the burned area. This fact emphasizes the important role which the burned area plays in the increased susceptibility of the burned rat and also demonstrates the restoration of resistance to *Ps. aeruginosa* in non-burned areas. Twenty-four hours after burning with steam the LD_{50} for rats injected intraperitoneally was 1.9×10^5, almost that of the normal rat.

Discussion. The most important observation in this work is the rapid reduction in LD_{50} caused by burning from more than 10^7 organisms to less than 10 within 2 minutes after burning. This increased susceptibility of rats to *Ps. aeruginosa* is probably associated with burn shock and trauma. Although data are not included, rats with 23% of the body surface burned did not show the marked susceptibility to *Ps. aeruginosa,* apparently indicating insufficient shock and trauma. There are few studies on the role of shock in lowering the host's defense to infection(7, 8). Miles(8) demonstrated that guinea pigs shocked by several methods were more susceptible to infection with *Staphylococcus aureus* for a short period than were normal guinea pigs. Studies with experimental animals exposed to irradiation or hemorrhagic shock have shown a reduction in normal host resistance(9,10). The striking reduction in resistance of burned rats to *Ps. aeruginosa* is probably due to alteration or impairment of normal host defense mechanisms adversely affected by the burn shock and trauma. One of the principal effects of burn shock is increased capillary permeability. Arturson(11) has shown, in burned rats, that capillary permeability is increased in non-burned areas as well as the burned area. Thus, a possible explanation for the increased susceptibility of

the burned rat to *Ps. aeruginosa* suggested by Arturson's study and work now in progress in this laboratory, is that increased capillary permeability permits bacteria to reach tissues which are normally protected by the impermeability of blood vessels to the passage of bacteria. Such tissues as muscle and brain, which have been shown to contain the test organism, along with the burned area may serve as reservoirs where the bacteria reproduce until sufficient in number to kill the animal. Experiments are being carried out on female rats, whose survival time is longer, to verify this observation. It is not appropriate to relate the findings reported here to clinical experiences with burned patients. Our present objective is to develop an understanding of the observations made in burned rats.

Summary. The LD_{50} of *Ps. aeruginosa* for extensively burned rats 2 minutes after burning, injected intraperitoneally or subcutaneously in the burned or non-burned area, was less than 10 organisms. The period of increased susceptibility existed for at least 24 hours when organisms were injected subcutaneously in the burned area but was less than 24 hours when organisms were injected intraperitoneally or subcutaneously in non-burned areas.

1. Rosenthal, S. M., *Public Health Rep.*, 1943, v58, 513.
2. Rosenthal, S. M., Lillie, R. D., Verder, E., *Fed. Proc.*, 1952, v11, 387.
3. Verder, E., Rosenthal, S. M., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 501.
4. Goshi, K., Cluff, L. E., Johnson, J. E., Conti, C. R., *J. Exp. Med.*, 1961, v113, 249.
5. Liedberg, C. F., *Acta Chir. Scand.*, 1960, v120, 88.
6. Reed, L. J., Muench, H. R., *Am. J. Hyg.*, 1938, v27, 493.
7. Miles, A. A., Niven, J. S. F., *Brit. J. Exp. Pathol.*, 1950, v31, 73.
8. Miles, A. A., *N. Y. Acad. Sci.*, 1956, v66, 356.
9. Frank, E. D., MacDonald, J. B., Palmerio, C., Schweinburger, F. B., Fine, J., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 394.
10. Miller, C. P., Hammond, C. W., Anderle, S. A., *J. Exp. Med.*, 1960, v111, 773.
11. Arturson, G., *Acta Chir. Scand.*, 1961, Suppl. 274.

Received July 19, 1963. P.S.E.B.M., 1964, v115.

Ability of Lupus Erythematosus Serum to Inactivate Bacteriophage ΦX174 Surviving Treatment with Specific Rabbit Antibody.* (28907)

BERNARD U. BOWMAN, JR.† AND ROBERT A. PATNODE
(Introduced by Martin M. Cummings)
Department of Microbiology, University of Oklahoma Medical Center, Oklahoma City

The possibility that both viral infectivity and neutralizing antibodies could co-exist in the same serum-virus mixture was suggested many years ago(1,2). Dulbecco *et al*(3) reported that excess antibody failed to neutralize completely the infectivity of either Western equine encephalitis (WEE) virus or poliovirus. Similar observations have been made in bacteriophage neutralization studies(4-6). Dulbecco's observations suggested to Herriott(7) that part or all of the residual infectivity might be due to infectious nucleic acid (INA). Studies in our laboratory have shown that phage escaping neutralization in the øX174-anti-øX174 system are able to infect protoplasts of *Escherichia coli* K12S which are normally resistant to intact øX174(8). These results suggested that the phage surviving neutralization might be due to a mechanism involving INA. Since Stollar *et al* (9) found that certain sera from patients with lupus erythematosus (LE) reacted with chemically isolated øX174 deoxyribonucleic acid (DNA), it seemed of interest to deter-

* Supported in part by USPHS Training Grant.
† Supported by USPHS Predoctoral fellowship. This paper is taken from a dissertation submitted to the Graduate School, Univ. of Oklahoma Med. Center, in May, 1963, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.