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.

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

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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-

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mine whether anti-DNA antibodies contained in LE sera might further inactivate bacteriophage øX174 surviving treatment with specific antibody.

Materials and methods. Phage. A highly purified lot of øX174 bacteriophage, prepared by rubidium chloride density gradient centrifugation according to the technique of Sinsheimer(10), was used throughout these studies. It contained only 114 S virions.

Specific antiserum. A single antiserum was prepared from blood taken by cardiac puncture from a rabbit 6 days after a second intravenous injection of approximately 1×10^{12} plaque-formers of øX174. This serum (S24) had a K value of about 3,000 minute $^{-1}$.

LE sera. Fifteen sera obtained from patients with LE were used in modified neutralization experiments described below. The source and description of these sera are presented in Table I.

Normal sera. Ten sera were obtained from normal persons and hospitalized individuals who did not have LE.

Neutralization experiments. In general, the standard bacteriophage techniques of Adams (11) were used. After varying periods of neutralization of øX174 by S24 at 37°C, the LE sera were tested for their ability further to inactivate the surviving phage. For this purpose, 0.1 ml volumes of the various LE sera were mixed with 0.9 ml volumes of the øX174-S24 mixture and incubation was continued. At 30-, 60-, 90- and 120-minute intervals samples were removed, diluted if necessary, and assayed for surviving phage. The 10 normal sera were similarly tested. Control reaction mixtures were prepared by mixing 0.9 ml volumes of the øX174-S24 mixture with either 0.1 ml of broth containing 1:100 normal rabbit serum (NRS) or 0.1 ml of a suitable dilution of S24. The concentration of S24 used was designed to yield, on dilution of 1:10, the same concentration present at the beginning of the neutralization period.

Results. The results of a typical experiment, using LE 8, are shown in Fig. 1. The initial phage concentration (P_o) was 3.28×10^7 plaque-formers ml^{-1} and S24 was used diluted 6×10^4 times. It can be seen that rate of neutralization of øX174 during the first 3 hours of neutralization was essentially exponential and decreased subsequently until, at 10 hours, it was approximately zero. At this point, addition of LE 8 resulted in an increase in the extent of neutralization approximately 10 times greater than that seen with control tubes containing broth or S24.

A second type of study was designed to determine whether LE serum would have any effect on øX174 not previously treated with S24. In a typical experiment, the P_o was 5.2×10^8 plaque-formers ml^{-1}. S24 was used at a dilution (1×10^4) which, in preliminary studies, gave a surviving fraction of approximately 1.22×10^{-5} after 4 hours of incubation at 37°C. At the end of 4 hours of incubation, a control suspension of øX174 containing no S24 was diluted 10^5 times so that its concentration of phage would be approximately the same as that in the reaction mixture containing S24. To 0.9 ml of the control phage suspension was added 0.1 ml of broth; to another 0.9 ml portion of the

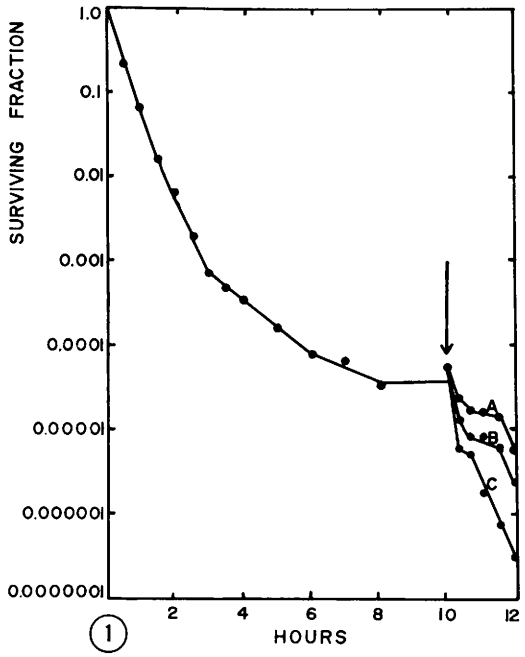
TABLE I. Lupus Erythematosus Sera Used in Neutralization Experiments.

Serum No.	Patient	Date patient was bled	Results of tests for antinuclear antibodies: Spot*	Results of tests for antinuclear antibodies: Complement fixation†
LE 1	S.J.	2/24/62	—	—
LE 2	N.P.K.	—	—	N
LE 3	L.B.	3/12/62	N	N
LE 4	N.P.K.	1/10/60	—	±
LE 5	B.M.	1/10/61	1:8	N
LE 6	J.K.	10/31/62	1:32	N
LE 7	H.H.	9/14/62	1:25	AC
LE 8	N.P.K.	11/ 7/62	—	N
LE 9	C.F.	12/12/62	1:256	AC
LE 10	J.K.	12/12/62	1:16	+
LE 11	C.A.	11/10/62	1:4	+
LE 12	J.K.	1/10/62	—	N
LE 13	K.P.	11/12/62	1:16	N
LE 14	R.G.V.	12/ 1/62	1:64	+
LE 15	J.K.	11/21/62	1:256	+

* Performed by Dr. George J. Friou. Results given as highest dilution of serum giving a positive test. N is negative test.

† Performed by Dr. George J. Friou, by method of Robbins *et al*(14). N is negative test; ± is doubtful test; + is positive test; AC is anticomplementary serum.

—, not recorded.



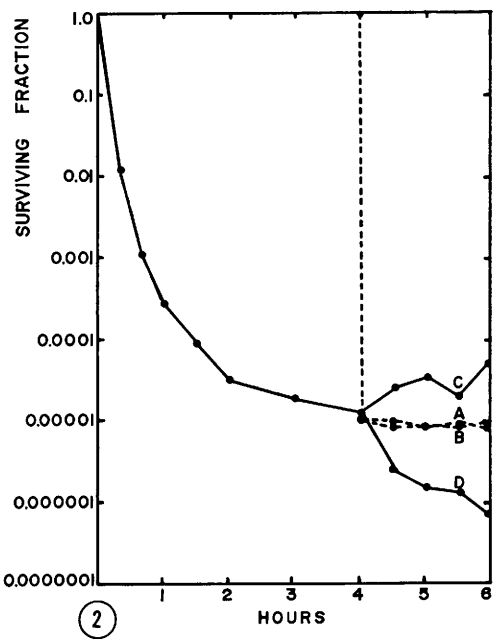


FIG. 1. Inactivation of surviving ΦX174 by LE 8. Incubated at 37°C in broth with 1:100 NRS. S24 was used diluted 6×10^4 times and P_o was 3.28×10^7 plaque-formers ml^{-1}. After 10 hr incubation (arrow), 0.9 ml samples of the reaction mixture received 0.1 ml broth (curve A); 0.1 ml S24 diluted 6×10^3 times (curve B); and 0.1 ml LE 8 (curve C).

FIG. 2. Inactivation of surviving ΦX174 by LE 8. Mixtures were incubated at 37°C in broth with 1:100 NRS. S24 was used diluted 1×10^4 times and P_o was 5.20×10^8 plaque-formers ml^{-1}. After 4 hr incubation the control mixture (without S24) was diluted $1:10^5$, as shown by vertical broken line, and 0.9 ml samples received 0.1 ml broth (curve A) and 0.1 ml LE 8 (curve B). After 4 hr incubation of the ΦX174b-S24 mixture (solid line), 0.9 ml samples received 0.1 ml broth (curve C) and 0.1 ml LE 8 (curve D).

control suspension was added 0.1 ml of LE 8. Two 0.9 ml portions of the phage-anti-phage reaction mixture were treated in exactly the same manner. The results presented in Fig. 2 show that LE 8 inactivated øX174 only if the latter was first pre-treated with S24.

All of the LE sera listed in Table I were tested for their ability to inactivate øX174 previously treated with S24. Only LE 2, 8, 9, 11 and 12 were found to be effective. The 10 normal sera were ineffective.

These results and those of the protoplast experiments(8) suggested that the surviving phage might be sensitive to the action of DNase. To test this possibility, pancreatic DNase (obtained from California Corp. for Biochemical Research) in distilled water or 0.05 M Tris buffer, pH 7.1, was incubated at a concentration of 0.25, 2.5, and 25 $\mu g\ ml^{-1}$ with control phage and with serum-treated phage at 37°C for intervals up to 120 minutes. Under these conditions, the enzyme had no effect on the infectivity of either the control phage or the phage which survived treatment with specific serum.

Discussion. The naturally occurring antibodies to nuclei, nucleoprotein, or DNA in serum of patients with lupus erythematosus (LE), have been studied intensively. Stollar and Levine(12), using quantitative complement fixation tests, described a reaction between DNA and the serum of a patient with disseminated LE. They found that thermally denatured DNA was a more reactive antigen than unheated DNA. Using complement fixation tests, Stollar *et al*(9) found that 11 of 37 samples of LE serum reacted with denatured DNA, whereas none of 124 sera from patients with other diseases and from normal persons reacted. They tested an LE serum with boiled and unboiled samples of DNA from bacteriophage øX174 and found that

both antigens fixed identical and relatively large amounts of complement. Similar results were obtained with another LE serum which failed to react with any other native DNA sample tested. Since the DNA of øX174 is known to be single stranded(10), and one LE serum reacted only with denatured DNA and with øX174 DNA, Stollar *et al* conclude that the anti-DNA antibodies in LE serum reacted more effectively with single stranded DNA.

The present studies were directed toward determining whether LE serum would inactivate the surviving bacteriophage øX174 in neutralization experiments. Of several LE sera found to have this ability, one in particular, LE 8, almost completely neutralized the residual infectivity of øX174 pre-treated with specific antiserum. Phage not previously treated with specific antiserum was not affected by LE serum. These results coupled with those on the ability of the surviving phage to infect protoplasts(8) suggests that part of the phage surviving neutralization by specific antiserum could have been physically altered. This surviving, infectious fraction could represent DNA in damaged capsids, partially or completely exposed, presumably as infectious nucleic acid (INA). The resistance of the serum-treated phage to the enzymatic action of DNase indicates that the material which was infectious for K12S protoplasts and sensitive to the LE sera is probably not free DNA. Possibly this material is DNA associated with some viral protein. Further, the exposed or free DNA would be infectious for *E. coli* by a mechanism similar to that involved in the transfer of DNA in transformation and would be resistant to inactivation by antiserum against intact phage. Support for this concept can be found in the results of experiments by Butler *et al*(13) showing inhibition of transformation in pneumococci by antipurinoyl antibody.

Summary. After 4 to 10 hours of incubation, essentially all the surviving phage in the øX174 phage-anti-phage system was sensitive to further inactivation by several sera from patients with lupus erythematosus (LE) but they were not sensitive to the enzymatic action of DNase. Phage not previously incubated with specific antiserum was resistant to LE serum and DNase. A hypothesis involving infectious nucleic acid as the underlying mechanism for this behavior is presented.

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