ficiently stable to last through the proliferative cycle of the cells, and intracellular degradation or protein turnover does not have a significant role in fixing the net rate of protein synthesis or in contributing appreciably to the composition and protein structure of the proliferating cells (*8*).

M. LIPKIN
T. P. ALMY
H. QUASTLER

Department of Medicine, Cornell University Medical College, 2nd (Cornell) Medical Division, Bellevue Hospital, New York, and *Department of Biology, Brookhaven National Laboratory, Upton, New York*

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Immunization as a Factor Affecting the Course of Septicemic Anthrax

Abstract. Immunization of guinea pigs with alum-precipitated antigen increased resistance approximately 1600 times. The growth rate of *Bacillus anthracis*, in the septicemic phase, was the same in both normal and immunized animals; however, the number of bacilli per milliliter of blood at death was decreased by 75 percent in immunized animals. Neither the enhancement of virulence by treatment with egg yolk nor the size of the challenge dose affected the growth rate or the number of bacilli per milliliter of blood at death. Mean time to death for the treatments tested varied from approximately 20 to 100 hours. Nonimmunized animals challenged with a strain of low virulence had a terminal number of bacilli per milliliter of blood and a growth rate in the blood the same as that for the highly virulent strain.

Anthrax is typified by an extremely rapid progress of disease, with symptoms and septicemia occurring some few hours before death of the animal. Bloom *et al.* (*1*) and Keppie *et al.* (*2*) state that the number of organisms per milliliter of blood appeared to be lower in partially immunized guinea pigs than in nonimmunized animals. This paper presents quantitative data regarding the in vivo rate of growth and the level of bacilli in the blood of immunized and normal guinea pigs during the septicemic stage of anthrax and notes the constancy of these observations regardless of certain treatment variations of the initial challenge dose.

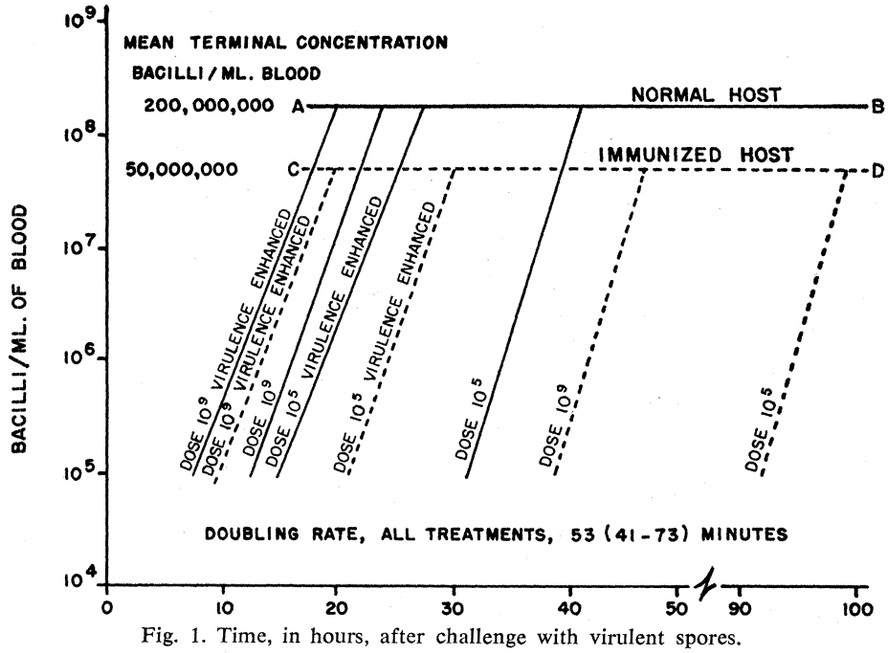

Fig. 1. Time, in hours, after challenge with virulent spores.

An experiment of four factors each at two levels was designed to investigate the septicemic phase of anthrax. These factors were host (immune or nonimmune), virulence (high or low), virulence-enhancement (enhanced or normal), and dose (10^9 or 10^5 spores); however, only the high dose level of the low virulent strain was used, because the low dose did not kill the host. Hartley strain guinea pigs, weighing 250 to 300 g, raised by the Fort Detrick animal farm were immunized by administration of 0.1 ml of a 1:10 dilution of antigen (*3*) given by intraperitoneal injection on days 1, 3, 5, 8, and 11. The animals were challenged on the 21st day with spores of either the virulent V1b strain or the low virulent 30R strain, a mutant of V1b. The immunity attained by this procedure, when the animals were challenged with spores of the virulent V1b strain, was approximately 1600-fold, that is, equal responses of immunized and control hosts were obtained by increasing the challenge dose of immunized animals 1600-fold. Virulence was enhanced by the addition of egg yolk medium to the inoculum (*4*). The measurement of virulence was based on a graded response method (*5*). After two initial experiments to establish techniques, groups of four immunized and two control animals were challenged with each of the treatment combinations, and tests were replicated on three occasions. The number of cells in the blood was determined quantitatively both by direct observation of stained slides, by the methods of Keppie *et al.* (*2*), and by a plate count assay method. The concentration of bacilli per milliliter of blood at the time of death is known as the terminal concentration. The factorial design was used to gain efficiency in regard to number of animals required for significant conclusions.

Data reported in a schematic graph (Fig. 1) are quantitative counts of bacilli observed on stained slides. For all treatment conditions the growth of bacilli in the blood, observed during approximately the 12 hours preceding death, was exponential, and all growth curves were parallel as shown in Fig. 1. The doubling rate of bacilli in the blood (apparent generation time) averaged 53 minutes (41 to 73 minutes for 95 percent confidence limit). The number of bacilli per milliliter of blood in the normal animals was constant for all treatment conditions (line *AB*, Fig. 1) and approximately four-fold higher than the average number found in the immunized host (line *CD*, Fig. 1). This difference is statistically significant and is based on 23 immunized and 15 control guinea pigs. Survival time in normal guinea pigs is considerably shorter than in immunized guinea pigs. Response of animals challenged with the 30R strain of low virulence was only partial; therefore, data on these

variables are incomplete. However, the constants of apparent generation time and terminal concentration appear to be equivalent to that observed with the virulent V1b strain, although it is noted that most of the animals responding to the 30R challenge were those challenged with virulence-enhanced spores.

Thus it is concluded that the growth rate in the septicemic stage is not affected by specific host resistance, by dose level (or, alternately, time of response), or by enhancement of virulence by treatment of spores with egg yolk, a treatment that in these tests gave a 120-fold increased virulence for guinea pigs. We conclude, also, that immunization significantly lowers the number of cells present in the blood stream at death when normal and immunized guinea pigs are compared, and that the disease caused by a strain of low virulence, once it is established as septicemic anthrax, is or may be equally as severe and dangerous as that caused by the highly virulent strain.

There is no evidence from these data that the host defenses are overpowered or change during the period of septicemic growth—otherwise the response slopes would not be linear. Growth, as indicated by increased cell number during this final stage of the disease, may be compared favorably with the growth rate in the logarithmic growth phase of culture in vitro in media such as tryptose or nutrient broth. Since the terminal concentration of bacilli per milliliter of blood is significantly lower in immunized animals than in nonimmunized hosts, it follows that, if these data are translatable to other animals, treatment will need to be initiated while the number of bacilli per milliliter of blood is much lower in the immunized than in the nonimmunized hosts. The critical level of organisms per milliliter of blood in the guinea pigs was reported by Keppie *et al.* (*2*) to be 1/300th of the terminal concentration, and after this no effective antibiotic cure can be predicted.

Since death by anthrax has been ascribed to a toxemia (*6*), and since toxin has been detected both in vivo and in vitro, our observations raise questions regarding the critical number of organisms per milliliter of blood in relationship to toxin produced in immunized as compared to normal animals. It is possible, since immunization prolongs the time from dose to septicemia, that more organisms and more toxin are produced before septicemia is detected, and therefore the animal dies with a lower level of organisms per milliliter of blood.

FREDERICK KLEIN
BILL G. MAHLANDT
RALPH E. LINCOLN
IRA A. DEARMON, JR.
ALBERT L. FERNELIUS
U.S. Army Chemical Corps, Fort Detrick, Frederick, Maryland

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Growth of Sporangiophores of Phycomyces Immersed in Water

Abstract. Sporangiophores of *Phycomyces* are capable of sustained growth when immersed in aerated water, and under these conditions they are negatively phototropic.

When immersed in water saturated with air at atmospheric pressure, sporangiophores will grow for many hours at the rate of 1.5 mm/hr. This rate is equal to one-half the growth rate in air (*1*). The reduction of the growth rate is not due to lack of oxygen in the ambient water, because increasing the oxygen tension in the water by (i) saturating the water with pure oxygen or by (ii) saturating the water at 0°C with pure oxygen, warming slowly to 22°C under 4 atm pressure of pure oxygen, and maintaining this increased pressure throughout the test has no effect on the growth rate. In air, if the oxygen tension is raised above 20 percent, there is no change in the growth rate of the plant (*2*). It appears then, that when the rate of oxygen supply is not limiting, 1.5 mm/hr is the growth rate in water, in contrast to 3.0 mm/hr in air. In either case, there is probably no significant oxygen gradient at the cell wall.

I have attempted to measure the oxygen consumption of the growing zone by Warburg manometric techniques, but have succeeded only in establishing an upper limit of about 0.5 mm^3/hr.

Specimens growing in water exhibit *negative* phototropic reactions. This may seem surprising in view of the fact that the refractive index of water (1.33) is slightly below that of the cell contents (1.35), and Buder's theory (*3*) would therefore predict a positive reaction. However, it may be presumed that internal absorption, estimated to be 10 percent by Delbrück and Shropshire (*4*), is sufficient to overcompensate the slight residual lens effect.

The possibility of growing specimens in aerated water opens the way to testing the effects of any water-soluble substrates or inhibitors on growth and reactivity. I have tested a number of enzymatic inhibitors but have not found any that would discriminate between growth and photoreactivity (*5*).

RICHARD BOND STIFLER*
California Institute of Technology, Pasadena

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* Present address: Department of Biology, Massachusetts Institute of Technology, Cambridge.

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