found that significant amounts of B_{12} binding power or Co^{60} appeared in the bile within one hour and that such excretion began almost immediately following intravenous administration. A possible mechanism of hepatic uptake and subsequent excretion into bile of intravenously administred IF or its complex has been discussed.

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Influence of Spermine and Related Substances on Susceptibility of *Bacillus anthracis* to Lysozyme.‡ (27052)

GEORGE G. WRIGHT*
The Sir William Dunn School of Pathology, University of Oxford

In complex media certain strains of *Bacillus anthracis* become susceptible to the action of lysozyme only when bicarbonate is present during growth(1). This requirement for bicarbonate was not demonstrable in chemically defined media; under these conditions the strains remained susceptible to lysozyme when bicarbonate was omitted. Investigation of the cause of this difference revealed that the yeast extract present in complex media contained a dialyzable factor that caused susceptible strains to become resistant. It was the activity of this factor that was overcome by addition of bicarbonate to the growth medium(2).

Identification of the factor in yeast and elucidation of its action are of particular interest because of the possibility that related mechanisms may be involved in the bicarbonate requirements for elaboration of capsular polypeptide and protective antigen by this organism(3,4). The present report presents evidence that spermine, spermidine, and certain diamines reproduce the effect of yeast extract in inducing resistance to lysozyme.

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* Permanent address: Fort Detrick, Frederick, Md.

Methods. The basal medium contained 2% Difco vitamin-free casamino acids, 0.04% Difco yeast extract, and 0.2% glucose. The small amount of yeast extract was sufficient to allow growth of the test organism in the absence of lysozyme but not in its presence. The medium was adjusted to pH 7.6 with sodium hydroxide and sterilized by filtration. Ultra-fine sintered glass filters were used for all sterilizing filtrations. Sterile sodium bicarbonate solution was added to the basal medium in the concentrations indicated.

The compounds tested were obtained from commercial sources, spermine and the diamines as hydrochlorides, spermidine as the phosphate, and agmatine and arcain as sulphates. They were dissolved in water and sterilized by filtration. Titrations were carried out in ¾" x 6" tubes provided with aluminum caps. Serial 2-fold dilutions of the substances tested were prepared in a volume of 0.5 ml. To each tube were added 0.5 ml of 1-1000 solution of crystalline lysozyme (Armour Laboratories) and 1.0 ml of basal medium. Controls without lysozyme, without test substance, and without inoculum were included in each titration.

The inoclum consisted of approximately 100 spores of strain B of *B. anthracis*, added

as one drop of a suitably diluted stock suspension. Strain B is a typical lysozyme-susceptible culture(2). The tests were incubated at 37°, and growth was recorded after 40-48 hours.

Results. Various diamines, polyamines, and related compounds were titrated for their activity in permitting growth of the B strain in the presence of 1-4000 lysozyme. Titrations were carried out in the presence of 2 concentrations of sodium bicarbonate: 0.0005*M* and 0.005*M*. In the complete absence of added bicarbonate irregular results were obtained, presumably because of variations in absorption of atmospheric carbon dioxide. Addition of 0.0005*M* sodium bicarbonate obviated this difficulty and allowed relatively reproducible titrations, although this low concentration doubtless reduced the activity of the compounds somewhat. The results are summarized in Table I.

TABLE I. Activity of Diamines and Polyamines in Allowing Growth of *B. anthracis* in Presence of 1-4000 Lysozyme.

Substance	Concentration allowing growth (μmoles per liter)	
	.0005*M* $NaHCO_3$	.005*M* $NaHCO_3$
1-3 propanediamine	60	>2500
Putrescine	120	>2500
Cadaverine	600	>2500
Spermidine	6	> 500
Spermine	3	> 500
Agmatine	400	—
Arcain	>500	—

It may be noted that spermine and spermidine were effective in very low concentrations, 1-3 propanediamine and putrescine were also active but less so than the polyamines, and cadaverine was less active than putrescine. Agmatine, the monoguanidyl derivative of putrescine, was less active than putrescine itself, and arcain, the diguanidyl derivative, was inactive at the concentrations tested. The activity of all the compounds was markedly inhibited in presence of 0.005*M* sodium bicarbonate.

Discussion. The evidence suggests that spermine and spermidine are responsible for the activity of yeast extract in inducing lysozyme resistance in *B. anthracis*. These substances are active in very low concentration, and their effect, like that of yeast extract, is antagonized by bicarbonate. Spermine was isolated from yeast extract(5), and *Saccharomyces cerevisiae* was shown to synthesize spermine and spermidine(6). Furthermore, none of the numerous growth factors, amino acids, and other substances that were tested possessed similar activity(2).

Spermine and related substances are widely distributed in plant and animal tissues(7,8) and are essential or stimulatory factors for growth of *Hemophilus parainfluenzae*(9), *Neisseria perflava*(10), a mutant of *Aspergillus nidulans*(11), *Pasteurella tularensis*(12), and *Lactobacillus casei*(13). Putrescine, 1-3 propanediamine, spermine, and spermidine are accumulated or interconverted by a variety of microorganisms(6). Gram negative bacteria evidently accumulated larger amounts of putrescine than gram positive organisms(14). The synthesis of spermidine from putrescine and methionine in *E. coli* has been elucidated (15) and oxidative degradation of the polyamines by *Pseudomonas aeruginosa* and by *Neisseria perflava* has been investigated(16, 17).

Despite considerable knowledge of the biosynthesis, interconversion, and degradation of spermine and related compounds, their essential metabolic functions remain unknown. Accordingly, no explanation for their effect on resistance to lysozyme in *B. anthracis* can be postulated with assurance. With respect to the relative activities of the various compounds, the present system is analogous to growth stimulation of *P. tularensis* and *L. casei*, in that spermine and spermidine are significantly more active than the diamines. Recently it has been demonstrated that in *Micrococcus lysodeikticus*, resistance to lysozyme is associated with an increased concentration of O-acetyl groups in the cell wall(18). Should resistance in *B. anthracis* be explicable on a similar basis, tentative inferences could be made regarding the mode of action of spermine and related substances.

A ready explanation is not available for the activity of bicarbonate in overcoming the effect of spermine and related compounds. No similar effect of bicarbonate with respect to the other biological activities of these substances appears to have been recorded. In preliminary experiments, addition of bicarbonate to the growth medium did not suppress the ac-

cumulation of spermine-like activity in culture filtrates of *B. anthracis*. However, the possibility has not been excluded that the active substance was initially present in the culture as an inactive carbamyl compound, which was hydrolyzed during removal of bicarbonate prior to testing. Reaction of putrescine with carbamyl phosphate has been demonstrated in the presence of yeast and mold enzymes(19). It is perhaps significant that arcain, the diguanidyl derivative of putrescine, was inactive in the present system; dicarbamyl derivatives of the active compounds have not yet been tested.

Summary. Spermine was active at a concentration of 3 μ moles per liter in allowing growth of a lysozyme-susceptible strain of *B. anthracis* in the presence of 1-4000 lysozyme. Spermidine, 1-3 propanediamine, putrescine, and cadaverine were respectively 1/2, 1/20, 1/40 and 1/200 as active as spermine. The activity of all of the substances was overcome by addition of 0.005*M* sodium bicarbonate to the medium.

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Multiplication of Echo 9 Virus in Suspensions of Human Leucocytes.* (27053)

ROBERT B. BERG† (Introduced by John P. Fox)
Division of Epidemiology, Public Health Research Institute of the City of New York, Inc.

Isolation of ECHO virus type 9 from the serum of 10 of 18 children(1) indicates that viremia occurs commonly in human infection with this agent. In attempting to investigate a possible role of leucocytes during ECHO 9 viremia, experiments were performed to determine whether human leucocytes *in vitro* were capable of supporting multiplication of the virus. A previous publication(2) reported the multiplication *in vitro* of measles virus in human and simian leucocytes.

Materials and methods. Human blood cell suspensions were prepared by a modification of a previously described method(2). Venous blood was withdrawn from a healthy donor whose serum neutralized 100 TCD_{50} of ECHO 9 virus in 1:16 final dilution. Heparinized blood was centrifuged at 1500 RPM for 10 minutes. Buffy coat was washed 3× and finally resuspended in medium 199(3) containing 20% calf serum, penicillin and streptomycin. Final medium was adjusted to pH 7.4

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† Present address: Dept. of Pediatrics, Beth Israel Hospital, Boston, Mass.