The growth of the *Shigella* was proportional (over a range of 0 to 1 mg/ml) to the concentration of pyridine-3-sulfonic acid in the liquid medium; maximal growth occurred at a concentration of approximately 1.0 mg/ml. On the other hand, 10 to 100 μg/ml of nicotinic acid yielded maximal growth. The minimal inoculum size that consistently initiated growth in media containing pyridine-3-sulfonic acid was approximately 1,000 cells. This was comparable to the inoculum size required to initiate growth in the medium containing nicotinic acid (Nakamura and Pitsch, Can. J. Microbiol. **7**:848, 1961).

The exact mechanism by which pyridine-3-sulfonic acid substituted for nicotinic acid is not known. One possibility is that the antimetabolite was desulfonated, and, subsequently, a carboxyl group added yielding nicotinic acid. Another possibility is that the pyridine-3-sulfonic acid was incorporated into the coenzyme without materially affecting its activity. It is also conceivable that pyridine-3-sulfonic acid induced a change in the biosynthetic pattern of the shigellae, modifying them in such a way that the organisms were capable of synthesizing nicotinic acid.

This work was supported by grants from the Research Corporation and the U.S. Public Health Service (E-3734). The senior author was a U.S. Public Health Service predoctoral Research Fellow (EF-10,159).

STUDIES OF BOVINE ERYTHROCYTES IN ANAPLASMOSIS

IV. Effects of Sonic Vibration on *Anaplasma* Complement-Fixing Antigen

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Received for publication 21 February 1963

Sonic vibration has been employed to liberate *Anaplasma marginale* from its intraerythrocytic environment for use in subsequent filtration studies to determine size range (Allbritton and Parker, Am. J. Vet. Res. **23**:809, 1962), and for the preparation of an agglutinating antigen (Ristic, J. Am. Vet. Med. Assoc. **141**:588, 1962). Its effects on the infectivity of the organism have also been described (Bedell and Dimopoullos, Am. J. Vet. Res. **24**:278, 1963).

The complement-fixing (CF) antigen of *A. marginale* is associated with infected erythrocytes (Gates et al., Proc. U.S. Livestock Sanit. Assoc., p. 105, 1954). Studies at this laboratory have been oriented to the purification and characterization of this antigen. It appeared desirable to employ sonic vibration to liberate the active material. During these studies, certain observations were made on the effects of sonic vibration on the CF antigenic activity of material prepared from infected erythrocytic stromata. The results indicated that sonic treatment can be useful, only if it is controlled, in the liberation of the CF antigen from infected erythrocytes.

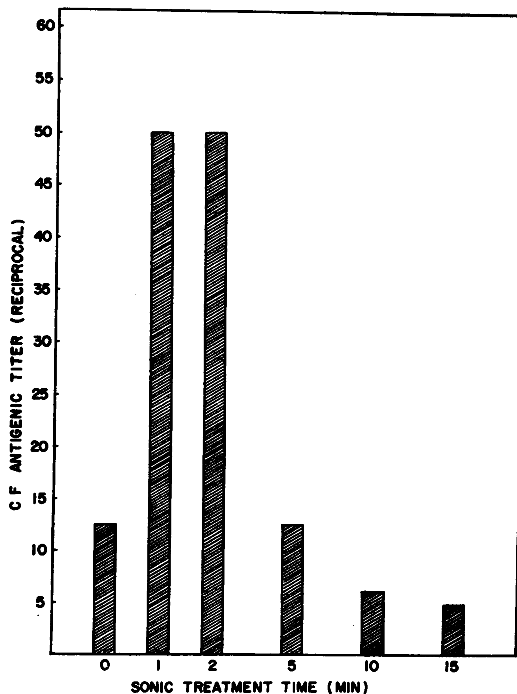

FIG. 1. *Effect of sonic vibration on the titer of complement-fixing stromatal antigen prepared from Anaplasma-infected erythrocytes.*

Standard *Anaplasma* antigens, consisting of lysed preparations of infected erythrocytes, were

obtained through the courtesy of the U.S. Department of Agriculture. Similar antigens, prepared in this laboratory, were also used. The samples were titrated according to protocol (*Manual for Conducting the CF Test for Anaplasmosis*, U.S. Department of Agriculture). Additional samples of CF antigens were subjected to sonic vibration at 17 to 20 C, using a 50-w, 9-kc oscillator (model S102A; Raytheon Magnetostriction Oscillator). The antigens were treated in 25-ml quantities for 1, 2, 5, 10, and 15 min. All samples were titrated for CF antigenic activity with appropriate controls. The results of the effects of sonic vibration on *Anaplasma* CF antigens are given in Fig. 1. Anticomplementary activity was not observed.

Exposure of the stromatal antigens to sonic vibration for periods of up to 2 min consistently resulted in over a fourfold increase in CF antigenic titer. After 5 min, the activity progressively decreased to slightly lower levels than the original titers. Controlled sonic treatment, therefore, may be of some importance in preparing superior CF antigens for anaplasmosis.

This work was supported in part by grant E-2250 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

CHEMICAL PROTECTION AND CHEMORESUSCITATION OF ULTRAVIOLET-IRRADIATED *SHIGELLA SONNEI*

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Received for publication 25 February 1963

Pyruvate, acetate, iodoacetate, sodium azide, urocanic acid, and metabolites of the tricarboxylic acid cycle have been shown to protect cells to some extent against the damaging effects of ultraviolet irradiation (Thompson, Mefferd, and Wyss, J. Bacteriol. **62**:39, 1951; Heinmets, J. Bacteriol. **66**:455, 1953; Ellison, Erlanger, and Allen, J. Bacteriol. **69**:536, 1955; Wainright and Nevill, J. Gen. Microbiol. **12**:1, 1955; Berger et al., J. Bacteriol. **65**:538, 1953; Leif and Hebert, Am. J. Hyg. **71**: 285, 1960; Heinmets and Lehman, Arch. Biochem. Biophys. **59**:313, 1955).

We found that adenylic acid, guanylic acid, uridylic acid, uridine, uracil, adenine, nicotinic acid, nicotinamide adenine dinucleotide, and adenosine triphosphate protected *Shigella sonnei* against the inactivating effects of ultraviolet light. These agents (purchased from Nutritional Biochemicals Corp., Cleveland, Ohio) were added to the washed standard-cell suspensions during the period of irradiation. An ultraviolet dose of approximately 969 ergs/mm^2 was applied to approximately 1.0×10^8 colony-forming organisms/ml. Protective action was determined by comparing the numbers of viable colony-forming cells on recovery plates after irradiating the *Shigella* in the presence and absence of the test compound. All the compounds were tested at 0, 10, 100, and 1,000 μg/ml. The protective agents were comparable in activity. For example, when adenylic acid (1,000 μg/ml) was present during the irradiation, 7.7×10^7 colonies/ml were recovered compared to 10^3 colonies/ml in the absence of this nucleotide.

Chemoresuscitation was studied by irradiating cell suspensions and incubating the cells for 24 hr in phosphate buffer (pH 7.2) containing the test compound at 37 C. This was followed by decimal dilution and plate counting. By comparing the numbers of colonies with controls which were incubated without the resuscitative substances, we observed that adenylic acid, uridylic acid, uridine, uracil, and adenosine triphosphate restored the viability of the irradiated cells. Certain workers have criticized this method of measuring reactivation, since they believed that "apparent" reactivation arises from the proliferation of survivors in the metabolite solution used for reactivation (Garvie, J. Bacteriol. **69**:393, 1955; Hurwitz, Rosano, and Blattberg, J. Bacteriol. **73**:743, 1957). In an attempt to overcome this criticism, we studied the effect of the resuscitative agents upon the cells. We compared numbers of cells that formed colonies when incubated in synthetic media containing the resuscitative agent

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