In vitro Sensitivity of Some Bacteria, their L Forms and Pleuropneumonia-Like Organisms to Antibiotics.*† (23667)

JOHN R. WARD,‡ SARABELLE MADOFF AND LOUIS DIENES
Departments of Bacteriology and Medicine, Mass. General Hospital, and Robert W. Lovett Memorial Foundation for Study of Crippling Diseases, Harvard Medical School, Boston, Mass.

Data on the sensitivity of pleuropneumonia-like organisms (PPLO) to various antibiotics are available in the literature(1,2). No such data have been available for the L forms of bacteria. Comparison of the two groups in this respect is of interest because they present many similarities, one of which is their marked resistance to penicillin. Such resistance is present in the L forms regardless of the sensitivity of the parent organism.

Materials and methods. The following bacteria and their L forms were studied: 3 Group A streptococci (AED, GL8, ADA),§ a Group C streptococcus (C), and *alpha*-hemolytic streptococcus (#23), a diphtheroid strain (NMI), 2 Proteus strains (52 and 18),¶ a Salmonella typhimurium (TM), 2 Vibrio strains (EZ5 and Nankin)‖ and an unidentified Gram negative bacillus (City).¶ The L forms were isolated on agar plates by exposure to penicillin and maintained by serial transfer on agar plates containing penicillin (1000 units per ml) until transplanted for antibiotic sensitivity. Eleven strains of pleuropneumonia-like organisms were studied. Strains Campo, 4330, 1454, 9358 and Washington** were isolated from human genitourinary tracts. Strain K5 was isolated from an epizootic of goats.†† Preston‡‡ was isolated from a spontaneous polyarthritis of rats and A28 from a rat in our laboratory. 'L', ALG and C15 are saprophytic strains isolated from compost and well water.§§ Sensitivities were determined on agar plates. Solutions of the commercial antibiotics bacitracin, erythromycin, neomycin sulfate, chloramphenicol, chlortetracycline HCl, tetracycline HCl, oxytetracycline HCl, streptomycin sulfate and penicillin potassium were preserved in the frozen state until used. Appropriate amounts of the antibiotics were added to the medium before pouring the plates. Final concentrations of the antibiotics were 2, 5, 10, 20, 50, 100, 200, 400, 600, 1000 and 2000 micrograms per ml (or units for bacitracin and penicillin). The test plates were inoculated with L forms and PPLO by agar blocks cut out from 48- to 72-hour cultures. This procedure gave more uniform growth for these organisms than transfer from broth cultures. The bacteria were transferred to the test plates from 18-hour broth cultures. Examination for bacterial growth was made at 24 and 48 hours. Growth of the PPLO and of the L cultures was examined at 48, 72 and 96 hours with the hand lens and checked by

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‡ Clinical and Research Fellow in Medicine, Mass. General Hospital, and Research Fellow, Harvard Medical School and Trainee, Nat. Inst. of Arthritis and Metabolic Diseases, U. S. P. H. S. Present address: Department of Medicine, College of Medicine, University of Utah.

§ Strains were those reported by Sharp, *et al.*(3).

‖ Proteus strain and the Vibrio strains were kindly supplied by Dr. Robert Tulasne, Strasbourg, France. Vibrio EZ5 was isolated from water. Nankin is an old strain of *Vibrio cholerae* from the collection of the Pasteur Institute.

¶ Strain 'City' has not been finally classified. It is a Gram negative bacillus which occurred as a contaminant on an agar plate and which produced L forms abundantly. These L forms are similar to those of Proteus, but the bacillus does not swarm and it gives a negative urease reaction.

** Received from Dr. Ruth G. Whittler, Washington, D.C.

†† Received from Dr. H. E. Adler, Davis, Calif.

‡‡ Received from Dr. Wm. S. Preston, Ann Arbor, Mich.

§§ Received from Dr. Otto Kandler, Munich, Germany.

means of stained agar preparations. Sensitivity of the organisms was expressed as the lowest concentration of the antibiotic inhibiting growth. At least 2 tests were made with every organism and antibiotic at different times using different batches of media and only slight differences were observed between the tests. The procedure employed determines the inhibition of growth. However, as the plates used for any experiment were prepared and inoculated simultaneously, the differences observed between the various organisms are thought to represent real differences in their behavior to the antibiotics.

Results. The results are presented in Tables I and II. A conspicuous resistance comparable to that observed with penicillin is not present with either the PPLO or the various L forms toward any other antibiotic tested. The sensitivity of the L forms isolated from various bacteria to different antibiotics is quite variable and usually parallels the sensitivity of the parent bacterium. An exception is bacitracin to which the L forms of streptococci and of Vibrio strain EZ5 are noticeably less sensitive than the parent bacterium. The enteric bacteria and their L forms are more resistant to the antibiotics than the other species studied and sometimes the bacteria, sometimes the L forms are slightly more resistant.

The sensitivities of PPLO strains isolated from man and animals are remarkably uniform. Their resistance to bacitracin and

TABLE I. *In Vitro* Sensitivity of the Bacteria and Their L Forms to Various Antibiotics. Concentrations are expressed as μg/ml (or units for bacitracin and penicillin) and represent lowest inhibitory concentration.

Organism		Bac.	Erythro.	Neo.	Chloro.	Tetra.	Oxytet.	Chlortet.	Strep.	Pen.
Streptococci										
ADA	Bact.	<2	<2	10	<2	<2	<2	<2	10	<2
	L	50	"	"	"	"	"	"	"	20,000
G1-8	Bact.	<2	"	"	"	"	"	"	"	<2
	L	50	"	<2	"	"	"	"	<2	10,000
AED	Bact.	<2	"	10	"	"	"	"	10	<2
	L	400	"	"	"	"	<5	"	"	10,000
C	Bact.	<2	"	100	5	5	5	5	"	<2
	L	50	"	<2	<2	<2	<2	<2	5	>20,000
#23	Bact.	<2	"	10	"	"	"	"	10	<2
	L	50	"	"	"	"	"	"	"	20,000
Diphtheroid										
NMI	Bact.	10	5	100	"	"	"	"	50	* 10
	L	20	<2	"	5	"	"	"	100	20,000
Proteus										
18	Bact.	400	400	200	100	100	200	200	200	* 20
	L	200	5	5	50	20	50	50	20	10,000
52	Bact.	100	100	50	50	200	400	200	50	* 50
	L	1,000	600	600	200	"	"	600	200	20,000
Salmonella										
TM	Bact.	"	"	2	10	20	20	20	20	* 50
	L	"	"	600	"	200	200	200	200	20,000
Unidentified										
City	Bact.	600	"	50	<2	50	100	50	50	* 50
	L	20	20	10	"	10	50	"	10	20,000
Vibrio										
EZ5	Bact.	<2	<2	<2	"	<2	<2	<2	<2	
	L	600	"	"	5	"	"	10	"	10,000
Nan.	Bact.	400	"	"	<2	"	10	20	20	
	L	600	"	"	5	"	<2	5	<2	10,000

Bac. = bacitracin; Erythro. = erythromycin; Neo. = neomycin; Chloro = chloramphenicol; Tetra. = tetracycline; Oxytet. = oxytetracycline; Chlortet. = chlortetracycline; Strep. = streptomycin; and Pen. = penicillin.

* These determinations were made at a different time from those relating to the L forms.

TABLE II. *In Vitro* Sensitivity of Some Pleuropneumonia-like Organisms to Various Antibiotics. Antibiotics and their concentrations same as in Table I.

Organism	Bac.	Erythro.	Neo.	Chloro.	Tetra.	Oxytet.	Chlortet.	Strep.	Pen.
Campo	600	200	10	10	<2	<2	<2	20	>20,000
4330	"	"	"	"	"	"	"	"	20,000
1454	"	"	"	"	"	"	"	"	"
9358	400	"	"	"	"	"	"	10	"
Washington	600	100	<2	<2	"	"	"	"	"
K5	"	200	10	"	"	"	"	20	>20,000
Preston	"	"	"	10	"	"	"	"	"
A28	"	"	20	"	"	"	"	"	20,000
Alg.*	"	<2	<2	<2	"	"	10	<2	"
C15*	"	"	"	5	"	"	"	"	"
"L"*	"	"	"	"	"	"	"	"	"

* Saprophytic strains.

erythromycin is fairly high, comparable to that of Gram negative bacteria. The saprophytic PPLO strains are sensitive to erythromycin and are more sensitive than the parasitic strains to neomycin, chloramphenicol and streptomycin also. On the whole, the sensitivity of PPLO to the various antibiotics is between the limits observed with the bacteria and their L forms. For example, the sensitivities of the L forms of the 2 Vibrio strains and of the saprophytic PPLO strains were similar toward all antibiotics tested. The PPLO, like the L forms, present an exceptional behavior toward penicillin only.

The similar reaction of the 2 groups to penicillin, like their morphological similarities, is probably the result of a common structural property, the lack of a rigid membrane comparable to the bacterial cell wall(3,4). Considering this and various biochemical observations, it has been inferred that the effect of penicillin may be to interfere with the production of the cell wall(3,5,6). On the other hand, the general similarity of behavior to various antibiotics of the PPLO, the L forms of bacteria and the bacteria themselves contributes to the concept that the PPLO are closely related to bacteria.

It is of interest that while penicillin makes possible the isolation of L forms, we had no such success with other antibiotics even when the difference between the sensitivity of the bacteria and their L forms is marked. For example, we were not able to obtain L forms with bacitracin from bacteria whose L forms grew well on media containing amounts of the antibiotic inhibitory for bacteria. Bacitracin also inhibited the development of L forms when it was added in subinhibitory doses to plates containing penicillin. Apparently, the bacitracin in concentrations which allow the growth of L forms interferes with the transformation of bacteria into such forms. The initial stage of the development of L colonies, the growth of small granules from the large bodies, was observed in typhoid bacilli in the presence of streptomycin and chloramphenicol, but development stopped at this stage (7). In contrast to these antibiotics, a few amino acids, especially glycine, are good producers of L forms, although there is no wide difference between the concentrations inhibiting growth of bacteria and L forms.

Summary. The sensitivity of PPLO strains of various origin and of several bacteria and their L forms were examined in the presence of 9 antibiotics. The sensitivity of L forms to the various antibiotics with the exception of penicillin was comparable to that of the parent bacterium. However, the L forms of streptococci and of one Vibrio strain were consistently less sensitive to bacitracin than their bacterial forms.

All PPLO strains, like the L forms, were highly resistant to penicillin. The sensitivities of the parasitic strains to the various antibiotics were uniform; they were markedly resistant to bacitracin and erythromycin, like some bacteria. The 3 saprophytic strains were noticeably less resistant than the parasitic strains to several antibiotics, especially to erythromycin. The sensitivities of all strains of PPLO, of bacteria and of L forms of these bacteria were on the whole comparable.

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Demonstration of Myosin in Human Striated Muscle by Fluorescent Antibody. (23668)

I. KLATZO, B. HORVATH, AND E. W. EMMART

Nat. Inst. of Neurol. Diseases and Blindness and Nat. Inst. of Arthritis and Metabolic Diseases, N.I.H., U. S. Department of Health, Bethesda, Md.

The antigenic property of myosin provides an opportunity to study this protein using immunological technics. Kesztyüs, Nikodemusz and Szilagyi demonstrated that myosin prepared from rabbit skeletal muscle was antigenic in dogs(1). Ebert has shown that chicken cardiac myosin produced antibodies in rabbits and that by the precipitin reaction it was possible to correlate the time of appearance of cross striations of the heart muscle with the presence of myosin(2). More recently Finck, Holtzer and Marshall(3) applied Coons' fluorescent antibody technic for localization of myosin in the chick muscle. This technic depends on selective binding of specific, fluorescent antibody to antigen present in tissue sections. The site of localization of antigenic substances within the morphological structures can be then demonstrated using fluorescence microscopy.

Our investigation represents a study of distribution of myosin in striated muscle by fluorescent antibody technic. It is the purpose of these studies on the localization of myosin in normal muscle to establish a basis of comparison for further study on the behaviour of myosin in diseased fibers and thus bring some insight into the problem of neuromuscular disorders.

Materials and methods. Preparation of antigen: Myosin was prepared from muscle of rabbit and goat and from human muscle, obtained on autopsy performed less than 2 hours post mortem. The muscle (*M. psoas* in most cases) was kept in the deep freeze until needed, *i.e.* for a period from several days to several weeks. The preparation followed closely the procedure of Szent-Gyorgyi(4). According to this method, the muscle is extracted with 0.6 M potassium iodide (KI) containing sodium thiosulphate after preliminary washing with water and with .05 M KCl to remove the muscle's adenosine triphosphate (ATP). The myosin was precipitated by diluting the extract with water to a KI concentration of .025 M. The precipitate was washed with .025 M NaCl and dissolved by adding NaCl to .5 M. The myosin was purified by 3 precipitations, and was dissolved in .5 M NaCl. Heavy impurities were removed by ultracentrifugation for 1 hour at 39,460 rpm. The myosin solution was passed through a Seitz filter before using it in precipitin reactions. The purity of the myosin preparations was ascertained in the ultracentrifuge. All operations were carried out in the cold. In addition, actin content was estimated by the method of Szent-Gyorgyi(4) from the change of the logarithm of the relative viscosity on addition of ATP. Most preparations contained less than 5% proteins other than myosin. Some myosin preparations were stored at −20°C in presence of .5 M NaCl and 50% glycerol. For immunizing rabbits and for precipitin reaction the glycerol was removed and the myosin precipitated by dilution or