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Effect of Antibiotics on L-Form Induction of *Neisseria meningitidis*

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Conditions have been developed that permit highly efficient conversion in vitro of all serogroups of *Neisseria meningitidis* to the L-form. During the course of the studies, optimal parameters for cultivation and propogation were determined and compared with those found in studies done by previous workers on meningococci. The plasma expander polyvinylpyrrolidone (PVP) (molecular weight, 40,000) has been incorporated into the medium as an osmotic stabilizer. A method is outlined for removing toxicity (due to impurities in PVP) reported in some earlier studies done in this laboratory. Light and scanning electron microscopy of the L-form inductants and of a stable L-form have shown characteristics distinctive among the strains observed. Finally, the effects of both cell wall and other antibiotics were studied. It was shown that cell wall antibiotics other than the commonly used potassium penicillin G could induce *N. meningitidis* to the L-form, using the PVP-stabilized medium base. Furthermore, the antibiotics vancomycin, colistin, nystatin, and trimethoprim, used at the concentrations found in commercially prepared Transgrow formulations, did not adversely affect meningococcal L-form induction. The system described herein will hopefully serve as an effective tool, specifically in the study of "aseptic" meningitis and possible meningococcal L-form etiology and, more generally, in evaluating the ability of bacterial L-forms to cause disease.

Since Klieneberger-Nobel (11) first described L-forms of *Streptobacillus moniliformis* in 1935, numerous studies of such bacterial variants have been undertaken. Recent interest in L-forms centers around their possible role in clinical disease states (3, 8-10, 12-14). In the case of meningococcal meningitis, an increase in recurrent disease cases, high carrier rate, and an unorthodox and problematical epidemiological picture suggest the possible involvement of L-form variants of *Neisseria meningitidis*. With this in mind, the present work was initiated to develop a more efficient system by which meningococcal L-forms could be studied.

Previous in vitro studies on the L-form induction of meningococci have used the penicillin gradient technique, originally described by Sharp (22) for induction of streptococci and subsequently adapted to the pathogenic *Neisseriae* species by Roberts (20, 21). More recently, Bohnhoff and Page (6) studied experimental infection of mice with both parent and L-form meningococci. Stewart and Wright (23, 24) worked on the adaptation of *N. meningitidis* L-forms to agar-free medium and their storage. All of these studies were done using the gradient induction techniques developed by Roberts, in which sucrose serves as the osmotic stabilizer for the L-form.

By incorporating polyvinylpyrrolidone (PVP) into the L-form induction medium as the osmotic stabilizer, Lawson and Douglas (16) have established cultural conditions that allow highly efficient conversion of *Neisseria gonorrhoeae* strains to the L-form, including several found to be noninducible in a sucrose-stabilized system. The present communication describes development of conditions that allow highly effective in vitro conversion of meningococci to L-forms.

MATERIALS AND METHODS

Organisms. Bacterial strains used in these studies were obtained from the Neisseria Repository, Naval Medical Research Unit no. 1, University of California, Berkeley. Twenty-four *N. meningitidis* strains were used in L-form induction studies (see Table 1). Scanning electron microscopic (SEM) studies were conducted on two of these strains. A stable *N. meningitidis* L-form (group A parent) was utilized for work with a defined liquid medium. This strain (M-1244L) was originally isolated and induced to the L-form by Bohnhoff and Page (6) and was adapted to broth growth by Pribnow et al. (18). Meningococcal strains were identified by: (i) Gram

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stain and morphology; (ii) sugar fermentation of glucose and maltose but not sucrose, lactose, levulose, and mannitol; (iii) positive oxidase reaction with *N,N*-dimethylphenylenediamine hydrochloride; and (iv) agglutination with specific rabbit antiserum (prepared by the Neisseria Repository). No serogrouping of the L-phase variant was performed by this laboratory. It had previously been adapted to stable L-form growth at the time of receipt by the Neisseria Repository.

Maintenance and storage of organisms. Cultures of parent meningococci were grown in Mueller-Hinton broth at 37 C. Routine dilutions were made in the same broth medium. Storage of meningococcal strains was accomplished by storage in tryptic soy broth (Oxoid) with 6% (wt/vol) lactose at −70 C or by lyophilization in the same suspending fluid. The meningococcal L-form was adapted to grow in tryptic soy broth with 5% (vol/vol) horse serum. Hence, stocks were maintained in this medium, and storage was performed as in the case of the parent organisms.

Media. Meningococcal growth and viable counts were obtained by culturing of the organisms on medium consisting of Mueller-Hinton medium (Difco, Detroit, Mich., or BBL, Cockeysville, Md.) with 5% (vol/vol) defibrinated sheep erythrocytes; this medium will be designated MHBA. The basic components of the L-form induction medium consisted of: (i) brain heart infusion broth with *p*-aminobenzoic acid (BBL, Cockeysville, Md.); (ii) 5% (vol/vol) heat-inactivated (56 C for 30 min) horse serum certified for growth of mycoplasma (Microbiological Associates, Richmond, Calif.); (iii) 1% agar (Difco) or Ionagar no. 2 (Consolidated Laboratories, Chicago Heights, Ill.) for solidification; and (iv) 100 units of potassium penicillin G per ml (Squibb). Stabilization of L-forms was achieved by incorporation of either 10% (wt/vol) sucrose (Mallinckrodt Chemical Works, St. Louis, Mo.) or 7 to 8% (wt/vol) PVP, pharmaceutical grade (Plasdone C, K-30, GAF Corp., New York, N.Y., or PVP-40, Sigma Chemical Co., St. Louis, Mo.), into the agar medium. These two media will be referred to, respectively, as sucrose L-medium (SLM) and PVP L-medium (PLM).

TABLE 1. *Characteristics of N. meningitidis strains studied*[a]

Serogroup	Strain no.[b]	No. of passes from original isolation	Drug susceptibility[c] (mg of sulfadiazine/100 ml)
A	M-135	>10	R 10
A	M-131	3	R 10
A	M-132	3	R 10
B	M-136	>10	S 0.1
B	M-290	3	R 10
B	M-414	3	R 10
C	M-137	>10	R 10
C	M-127	unk	S 1
C	M-299	unk	R 10
D	M-623	unk	S 0.1
D	M-618	unk	S 0.1
D	M-1057	unk	S 0.1
E	M-112	4	S 1
E	M-138	3	S 10
E	M-845	4	S 1
X	M-405	>10	S 0.1
X	M-569	unk	S 0.1
X	M-429	unk	S 1
Y	M-406	unk	R 10
Z	M-407	unk	R 10
Z	M-102	4	S 1
Z	M-2497	unk	S 0.1
W-135	M-603	unk	S 5
29E	JM-521	unk	S 1
A-L	M-1244(L)	unk	R 10

[a] unk, Unknown; R, resistant; S, susceptible.
[b] Neisseria Repository strain numbers.
[c] Unless otherwise noted, all strains susceptible to ampicillin, cephalothin, chloramphenicol, erythromycin, kanamycin, penicillin G, streptomycin, and tetracycline at concentrations used with the BBL Antibiotic Sensidisk system.

L-form induction procedures. Lyophilized parent strains of *N. meningitidis* were reconstituted into Mueller-Hinton broth. Dilutions of this concentrated suspension were prepared and allowed to incubate at 37 C for 12 to 24 h. Freshly poured agar plates were inoculated with appropriate samples of the freshly grown cultures and spread evenly with a glass rod. Induction media plates were incubated at 36.5 C for 4 to 6 days in a moist, air-tight chamber flushed periodically with a 10% CO_2-90% air gas mixture. Viable count plates were incubated for 1 to 3 days under similar conditions.

Microscopy. Sterile membrane filters of 0.45-μm pore size (Millipore Corp., Bedford, Mass.) were placed on freshly prepared PLM. Appropriate dilutions of parent meningococci were inoculated onto the membrane filters and the surrounding agar matrix. Plates were then incubated for 6 days at 36.5 C under the conditions specified above. SEM specimens were fixed and otherwise prepared according to the methods developed by Bibel and Lawson (4, 5). Examination of SEM samples was performed with a Cambridge Instrument Co., Ltd., Stereoscan electron microscope, operated at a voltage of 20 kV.

Antibiotic studies. These plates were prepared with varying concentrations of several antibiotics (see Tables 3–6). Accurate dispensing of the agar base medium, necessary for maintenance of proper antibiotic concentrations, was achieved by use of a Cornwall pipetting device. Meningococci and the stable L-form variant were grown in Mueller-Hinton broth (with supplemental horse serum in the case of the latter) for 18 to 24 h before inoculation.

RESULTS

L-form induction. Table 2 summarizes the induction frequencies obtained on SLM and PLM. Potassium penicillin G was incorporated into the media in amounts sufficient to yield final concentrations of 100 U/ml and served as the L-form inducing agent in both cases. The horse serum concentration used was varied be-

TABLE 2. *L-form induction of N. meningitidis strains*

Serogroup	Strain no.	% Induction to L-form[a, b]	
		SLM	PLM
A	M-139	0.66	7.4
A	M-139		5.5
A	M-132		20.8
B	M-136	1.5	24.6
B	M-290		21.0
B	M-414		65.0
C	M-137	25.0	80.0
C	M-127		6.3
C	M-299		68.4
D	M-623		0.4
D	M-1057		0.6
D	M-618		40.0
Y	M-112	50.0	66.7
Y	M-138		65.0
Y	M-845		44.5
Y	M-406	0.67	50.0
X	M-405		37.4
X	M-569		85.7
X	M-429		82.0
Z	M-407		0.0[c]
Z	M-102		100.0
Z	M-249		80.0
29E	M-603	0.38	90.0
W-135	M-521	0.07	20.0

[a] Percent induction to L-form = L-form frequency = (total L-form PLM or SLM colony-forming units per milliliter/total parent colony-forming units per milliliter) × 100.

[b] In the presence of potassium penicillin G, 100 U/ml.

[c] Did not induce to the L-form.

tween 1.0 and 20% (vol/vol) in the PLM system. Induction frequencies obtained with 5.0% (vol/vol) horse serum were higher than those obtained at lower serum concentrations, but further increases did not appreciably affect induction. Previous in vitro induction systems for the *Neisseriae* species (6, 7, 17, 20, 21, 23, 24) used candle jars to provide extra CO_2. Lawson and Douglas (16) have demonstrated that gonococcal L-forms can be induced at frequencies up to 100% under conditions in which a 10% CO_2-90% air mixture was used. Consequently, meningococcal L-form induction was tried with five different gaseous mixtures (air alone, candle jar, 5% CO_2-95% air, 10% CO_2-90% air, and 15% CO_2-85% air). Under strictly aerobic conditions without supplemental CO_2, no induction to the L-form occurred with any of the five strains tested. With candle jars (approximately 2 to 3% CO_2) and with the 5% CO_2-95% air mixture, two strains induced at levels under 0.015%, whereas the remaining strains tested did not induce at detectable levels (under 0.000001%). With higher concentrations of CO_2, much more favorable results were achieved. The data presented in Table 2 are averaged values obtained with the higher CO_2 concentrations, since no detectable differences could be shown between results obtained with 10 or 15% CO_2. Furthermore, some enhancement of L-form induction in the SLM system was seen when the latter gas mixtures were used instead of candle jars. The frequencies obtained with SLM were higher than those reported by others (21, 23).

Osmotic stabilization. Table 2 clearly shows that the incorporation of PVP as an osmotic stabilizer resulted in much higher L-form induction frequencies than previous workers have achieved with meningococci using sucrose-stabilized systems. However, not all samples of PVP were equally effective. Lawson and Douglas (16) originally used Plasdone C, K-30 (GAF), in their work with gonococci. Subsequent investigation revealed that PVP-40, pharmaceutical grade (Sigma), was superior to the GAF product in the PLM system. However, new batches of PVP-40 were found to be somewhat inhibitory even for growth of meningococci. This toxicity was not apparent for the initial gonococcal L-form experiments but was reported by Bacigalupi and Lawson (2) in their defined L-phase induction medium studies.

Accordingly, steps were undertaken to eliminate this toxicity. Ashwood-Smith and Warby (1) have shown toxicity in untreated Plasdone C, used as a cryoprotective agent for bacteria and erythrocytes. A modification of their technique was used. We prepared a 30% (wt/vol) solution of PVP-40 in distilled, deionized water. A batch dialysis of the PVP solution was then performed against carboxymethyl-cellulose (0.70 meq/g capacity, coarse grade) and diethylaminoethyl-cellulose (0.93 meq/g capacity, coarse grade). Both ion exchangers were prepared at a concentration of 5 g/10 liters of deionized water. This was followed by further dialysis against frequent changes of deionized water. All procedures were carried out at 4 C. The resultant solution had effective PVP concentrations ranging from 7 to 10% (wt/vol), measured by the method of Ashwood-Smith and Warby (1). The base constituents of PLM were then added and the medium was autoclaved.

Group D growth and inducibility. Two of the three group D meningococci induced at levels

below 1.0% in the PLM system. It was found that initial viable counts recovered from lyophilized cultures were lower among all the group D organisms than among members of other serogroups, and their growth on noninduction media was slower (at least 2 days of incubation at 36.5 C was necessary, as opposed to 24 to 36 h). Furthermore, the L-forms that developed were very small and showed granular morphology.

Group Z prototype strain induction. One of the 24 strains tested (M-407, group Z) failed to induce to the L-form in the PLM system. Three different forms of penicillin were tried in attempts to produce L-forms (potassium penicillin G, ampicillin, and carbenicillin). These were incorporated into the induction media (SLM and PLM) at four different concentrations (1, 10, 100, and 1,000 U of potassium penicillin G per ml and 1, 10, 100, and 1,000 μg of each of the other two derivatives per ml). Breakthrough occurred on the plates with 1 μg of ampicillin per ml, but no L-form induction was ever detected.

Colonial morphology and microscopy. General colonial morphology of the induced L-forms of meningococci was typical of such cell wall-less variants. However, strain and possibly serogroup variation was noted among the L-form colonies induced, especially on PLM.

Bibel and Lawson (4) have reported that differences in streptococcal L-form colonial morphology were enhanced when the organisms were grown on membrane filters (Millipore Corp.), according to the method of Wyrick and Gooder (25). Samples of parent group A and group Y meningococci were inoculated onto membrane filters (0.45 μm, Millipore Corp.) laid on the surface of PLM. Growth was sufficient after 5 days of incubation to allow examination. The colonies were fixed, dried using the critical point method, and coated with gold in a vacuum evaporator. They were then examined with the scanning-beam electron microscope (4, 5, 12). Figure 1 is a SEM photograph (×10,000) of the edge of a group A meningococcus L-form colony; on the left, one can see detail of the membrane filter. The colony consists of mainly pleomorphic large bodies about 2 μm in diameter. At higher magnification, ×22,000 (Fig. 2), granules of 0.2 μm in diameter can be detected

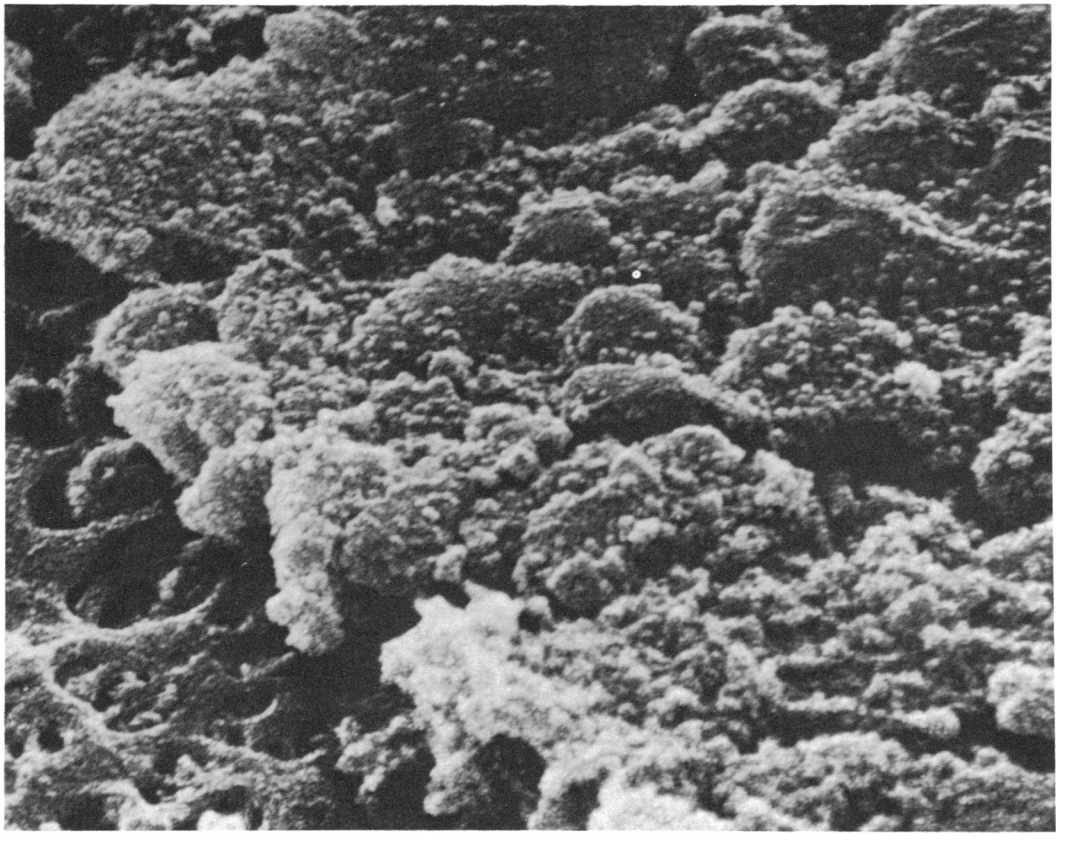

FIG. 1. *N. meningitidis, serogroup A, M-139; SEM, × 10,000; induced L-form colony.*

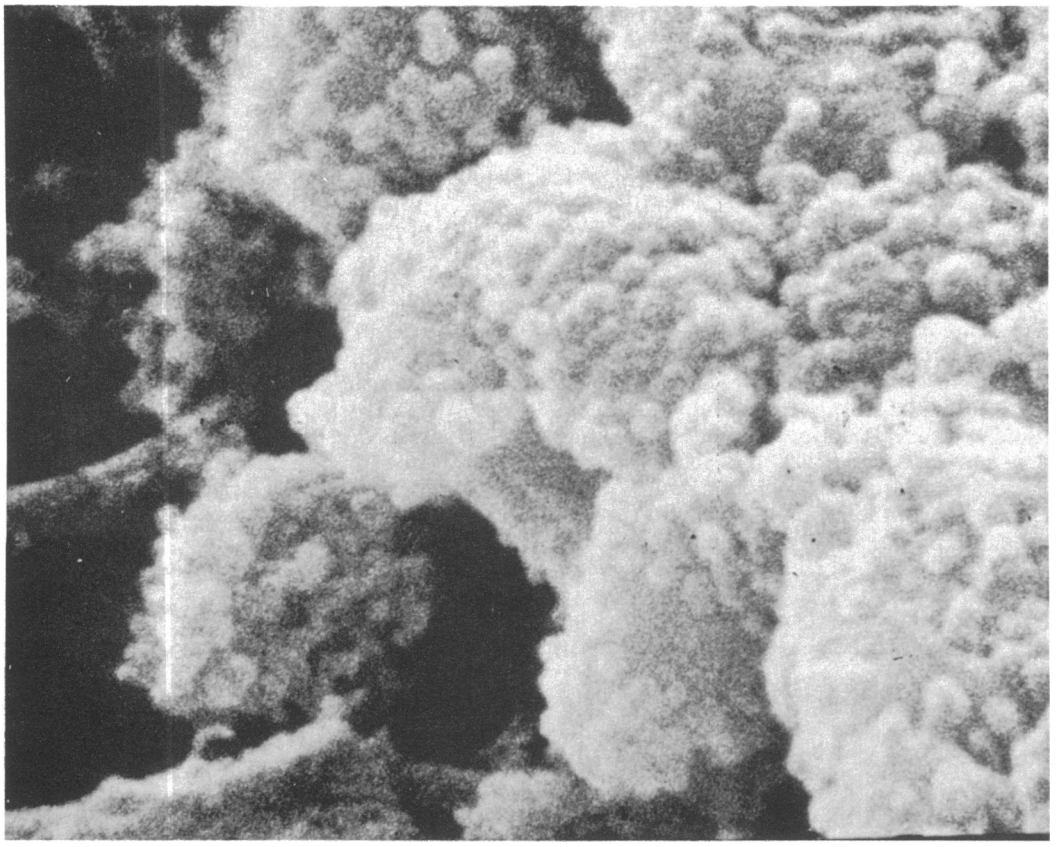

FIG. 2. *N. meningitidis, serogroup A, M-139; SEM, ×22,000; induced L-form colony. Note blebs and granular material on the surface of the large bodies.*

covering the entire surface of the larger bodies; again, note that the surface of the membrane filter (seen on the left border of Fig. 2) does not contain any granules. The group Y L-form colony, shown at ×5,700, could easily be distinguished from that of the group A meningococcal L-form (see Fig. 3). Figure 4, a closer view of the group Y colony (×20,000), reveals the presence of very fine filamentous material and ovoid large bodies about 2.5 μm in diameter. The small blebs and granular material seen in the previous example are also seen here.

The remaining micrograph (Fig. 5) shows the colonial morphology of a stable group A meningococcus L-form. Clearly, this organism lacks the small granules seen with the freshly induced L-form colonies. Large bodies range from 1.0 to 5.0 μm in diameter and are often pitted. Close examination reveals much cellular collapse and empty membranous debris. Such large, empty shells have also been noted when examining wet mounts of both broth- and agar-grown colonies of the stable, broth-adapted L-phase variant. Magnification in this photograph was ×10,000.

Antibiotic studies. After establishing our penicillin induction technique, the effect of other antibiotics on L-form induction of meningococci was evaluated. Table 3 shows the effect of two antibiotics not in the penicillin group. With cephalothin, L-form induction occurred both with and without penicillin. With the stable L-form culture, excellent growth was achieved at concentrations of cephalothin up to 100 μg/ml. In contrast, with novobiocin, no L-form induction occurred with or without penicillin. The data presented with the group C meningococcus as parent were typical of results obtained in induction experiments carried out with representatives of each serogroup. Furthermore, growth of the stable L-form was affected in a similar fashion to that of the bacterial form (Table 4).

The antibiotic studies were concluded by testing the effects of vancomycin, colistin, nystatin, and trimethoprim, used in selective pri-

mary isolation media for the pathogenic *Neisseriae*. It has been shown by Bacigalupi (personal communication) that these antibiotics, used in the concentrations found in Transgrow, inhibited the L-form induction process in the gonococcus. The source of inhibition of *N. gonorrhoeae* was vancomycin; this has also been reported for some gonococcal parent strains by Reyn and Bentzon (19). In contrast, vancomycin has no significant inhibitory effect on L-form induction of the meningococcus (Table 5). Table 6 shows that growth of the stable L-form was also unaffected. Similarly, no inhibition of either L-form induction or L-form growth was ever observed with colistin, nystatin, or trimethoprim (Tables 5 and 6).

DISCUSSION

In recent years, investigators have increasingly considered the involvement of bacterial L-forms in certain chronic disease or carrier states (3, 8–10, 12–15). Studies on the meningococcal L-form and L-forms of other pathogenic *Neisseria* species have been limited in the past by the relatively difficult and painstaking techniques required for successful production and propagation and the resultant poor L-induction frequencies. Previously reported systems have always used sucrose as the stabilizing agent for the L-variants. This paper presents a system for the production and propagation in the presence of penicillin of these L-forms by using the plasma expander PVP as the stabilizer. The induction frequencies obtained with this system are significantly higher than those achieved in past investigations on *N. meningitidis*.

The optimal growth requirements of *N. meningitidis* L-variants were found to differ somewhat from those reported by Roberts (20, 21). Growth of the L-form was found to be very poor in the commonly used candle jars (unlike that of the parent meningococci). Highly successful L-form induction required an atmosphere with CO_2 concentrations of 10% or higher. These conditions were also found to increase the frequency of L-form induction in the sucrose-stabilized medium used as our control system.

Lawson and Douglas (16) and Bacigalupi and

FIG. 3. *N. meningitidis, serogroup Y, M-406; SEM, ×5,700; induced L-form colony.*

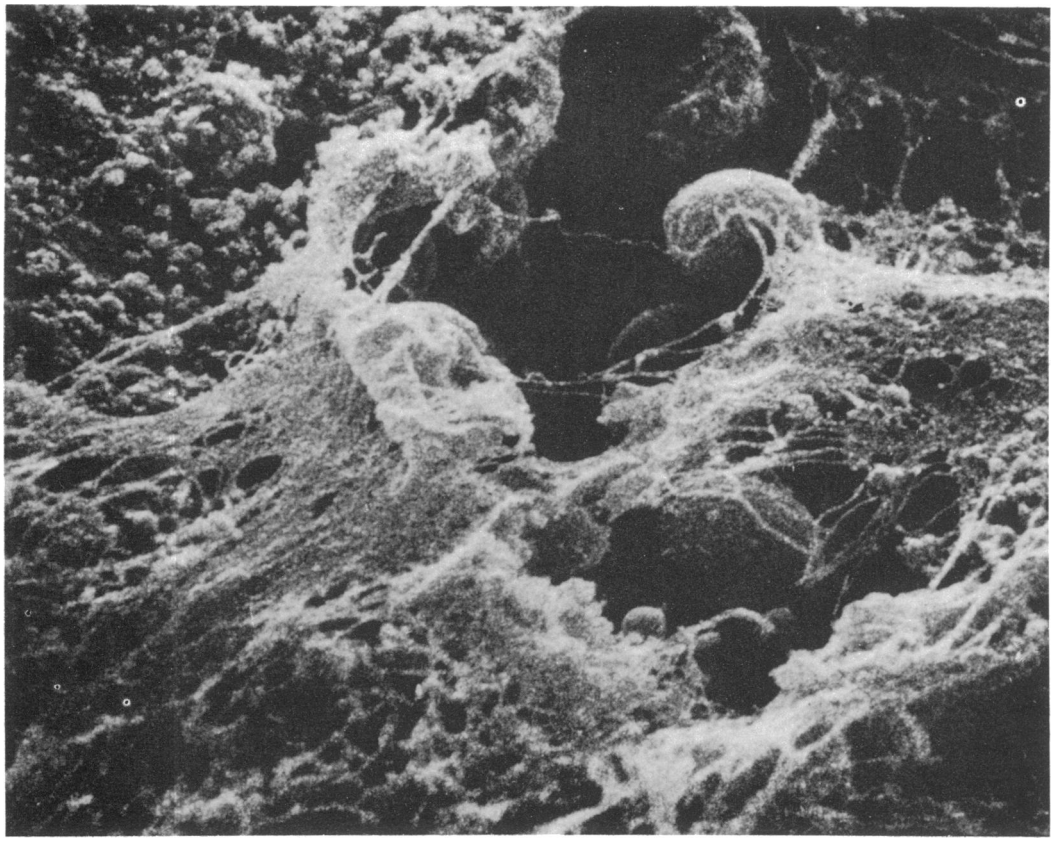

FIG. 4. *N. meningitidis, serogroup Y, M-406; SEM, × 23,000; induced L-form colony. Note appearance of very fine filamentous material and surface blebs.*

Lawson (2) have shown that PVP serves as an excellent osmotic stabilizer for gonococcal L-form induction work. The toxicity problem reported in this paper was not encountered by Lawson and Douglas working with the gonococcus in a complex gonococcal induction system. However, it is clearly evident both in the defined gonococcal system and in the work presented here. Furthermore, preliminary results indicate that clinical isolates of *N. gonorrhoeae* are similarly susceptible to PVP toxicity. Toxic samples of PVP apparently contain a wide range of molecules (molecular weight, 1,000 to 200,000); Ashwood-Smith and Warby (1) have proposed that the presence of low-molecular-weight acidic material in commercially available PVP was responsible for toxicity to their pseudomonad. Because of the strong autolytic enzyme systems present in the meningococcus and the lack of a rigid cell wall in the L-form their susceptibility to these toxic molecules may be much more evident than found with other organisms.

Roberts (20) found that L-inductants from different parent strains and different L-forms from the same parent strain vary; again, only partial agreement can be expressed here. Colonial morphology of L-forms induced from the same parent usually appeared the same and could almost be called characteristic of that particular strain when viewed under a dissecting microscope. However, inducibility of particular strains did vary. Group D parent organisms grew at a much slower rate than any other meningococci tested. It was consequently much more difficult to produce L-forms from these strains. Furthermore, the induced group D L-colonies remained very small, even after 10 days of incubation. One strain of meningococcus tested, prototype group Z, strain M-407, failed to induce to the L-form in our PLM system. Perhaps the use of other cell wall antibiotics, such as cephalothin, will permit induction of this strain. Interestingly, we noted that differentiation between two strains of meningococci of separate serogroups was possible with

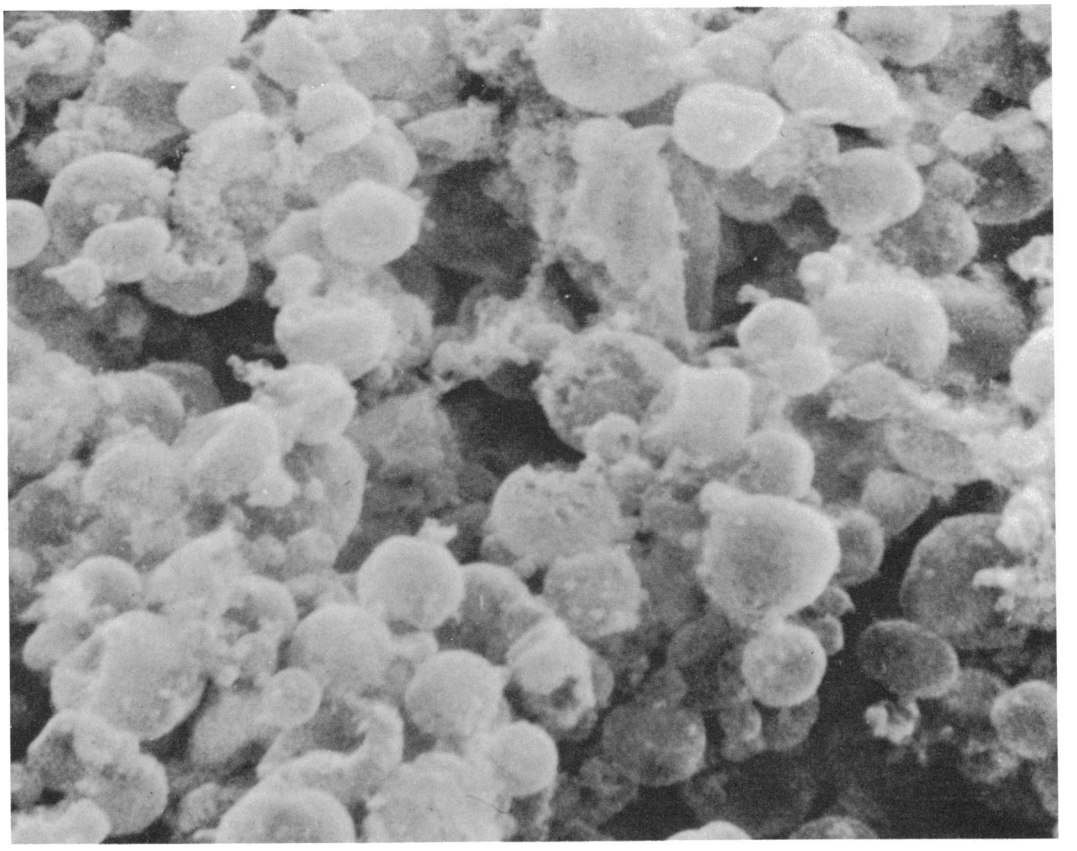

FIG. 5. *N. meningitidis, stable L-form, serogroup A, M-1244L; SEM, ×10,000.*

TABLE 3. *Effects of cephalothin and novobiocin on parent growth and L-form induction*[a]

Antibiotic	Concn (μg/ml)	Parental growth[b]	L-form induction[b]	
			Without penicillin	With penicillin[c]
Control (none)	NA	4+	NA	4+
Cephalothin	5.0	−	3+	2+
	10.0	−	4+	2+
	50.0	−	4+	2+
	100.0	−	4+	2+
	250.0	−	4+	1+
	500.0	−	4+	−
Novobiocin	1.0	4+	−	−
	2.5	2+	−	−
	5.0	1+	−	−
	10.0	−	−	−
	15.0	−	−	−
	20.0	−	−	−
	200.0	−	−	−

[a] +, Greater than 2.5×10^7 colony-forming units (CFU)/ml; 3+, greater than 10^7 CFU/ml; 2+, greater than 5×10^6 CFU/ml; 1+, greater than 10^5 CFU/ml; +, greater than 10^4 CFU/ml; −, no growth; NA, not applicable.

[b] *N. meningitidis*, group C M-137.

[c] In the presence of potassium penicillin G, 10 U/ml.

TABLE 4. *Effects of cephalothin and novobiocin on growth of a stable L-form of N. meningitidis*[a]

Antibiotic	Concn (μg/ml)	L-form growth[b]	
		Without penicillin	With penicillin[c]
Control (none)	NA	4+	4+
Cephalothin	5.0	4+	4+
	10.0	4+	4+
	50.0	4+	3+
	100.0	2+	2+
	250.0	2+	±
	500.0	1+	±
Novobiocin	1.0	3+	3+
	2.5	2+	2+
	5.0	1+	1+
	10.0	−	−
	15.0	−	−
	20.0	−	−
	200.0	−	−

[a] 4+, Greater than 2.5×10^7 colony-forming units (CFU)/ml; 3+, greater than 10^7 CFU/ml; 2+, greater than 5×10^6 CFU/ml; 1+, greater than 10^5 CFU/ml; ±, greater than 10^4 CFU/ml; −, no growth; NA, not applicable.

[b] *N. meningitidis*, group A, stable L-form, M-1244L.

[c] In the presence of potassium penicillin G, 10 U/ml.

TABLE 5. *Effects of VCNT inhibitor antibiotics on parental growth and L-form induction of N. meningitidis*[a]

Antibiotic	Concn (mg/ml)	*N. meningitidis* group A, M-139		
		Parent growth	L-form induction[b]	L-induction frequency[c]
Control (none)	NA	4+	3+	12.8
Vancomycin	0.5	4+	3+	12.0
	1.0	4+	3+	9.0
	2.0	4+	2+	8.3
	3.0	4+	3+	8.0
	5.0	4+	3+	8.75
	10.0	4+	3+	10.0
Colistin	1.0	4+	2+	4.9
	2.5	4+	2+	4.7
	5.0	4+	2+	5.0
	7.5	4+	2+	5.0
	10.0	4+	2+	5.0
Nystatin[d]	2.5	4+	2+	5.4
	5.0	4+	2+	5.3
	12.5	4+	2+	6.1
	50.0	4+	2+	2.3
Trimethoprim	1.0	4+	3+	9.6
	2.0	4+	3+	10.8
	3.0	4+	2+	5.0
	5.0	4+	2+	4.8

[a] 4+, Greater than 5×10^8 colony-forming units (CFU)/ml; 3+, 5×10^7 to 5×10^8 CFU/ml; 2+, 10^7 to 5×10^7 CFU/ml; NA = not applicable.

[b] In the presence of potassium penicillin G, 10 U/ml.

[c] Percent = (L-form colony-forming units per milliliter/parent colony-forming units per milliliter × 100.

[d] Nystatin concentrations expressed as units per milliliter.

TABLE 6. *Effects of VCNT inhibitor antibiotics on the growth of a stable L-form of N. meningitidis*[a]

Antibiotic	Concn (μg/ml)	*N. meningitidis* L-form growth, group A, M-1244L	
		Without penicillin	With penicillin[b]
Control (none)	NA	2+	2+
Vancomycin	0.5	2+	2+
	1.0	2+	2+
	2.0	2+	2+
	3.0	2+	2+
	5.0	2+	2+
	10.0	2+	2+
Colistin	1.0	2+	2+
	2.5	2+	2+
	5.0	2+	2+
	7.5	2+	2+
	10.0	2+	2+
Nystatin[c]	2.5	2+	2+
	5.0	2+	2+
	12.5	2+	2+
	50.0	2+	2+
Trimethoprim	1.0	2+	2+
	2.0	2+	2+
	3.0	2+	2+
	5.0	2+	2+

[a] 4+, Greater than 5×10^8 colony-forming units (CFU)/ml; 3+, 5×10^7 to 5×10^8 CFU/ml; 2+, 10^7 to 5×10^7 CFU/ml; NA, not applicable; VCNT, vancomycin, colistin, nystatin, and trimethoprim.

[b] In the presence of potassium penicillin G, 10 U/ml.

[c] Nystatin concentrations expressed as units per milliliter.

the use of SEM; Bibel and Lawson (5) reported a similar variation among streptococcal L-form colonies grown on membrane filters (Millipore Corp.).

Barbuti (3) has investigated the possibility that *N. meningitidis* L-forms are responsible for "aseptic" purulent meningitidis cases. However, he used sucrose-stabilized media in attempts to isolate the organisms and failed. He further does not include any data on the effect of vancomycin, colistin, nystatin, and trimethoprim on the meningococcal L-form. In this communication, commercial vancomycin, colistin, nystatin, and trimethoprim were used without reducing recovery of L-forms of the meningococcus. The ability to induce and propagate L-forms of *N. meningitidis* in the presence of antibiotics known to affect peptidoglycan biosynthesis will hopefully allow workers to efficiently and effectively study the meningococcal L-form and evaluate its possible role in persistence of infection, treatment failure, and in aseptic cases of meningitis.

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