membrane(s) of one or more of the intracellular particles.

Finally, other aspects merit consideration with respect to the requirement for high solute concentration. For example, the function could be to stabilize cellular deoxyribonucleic acid, ribonucleic acid or protein(s). Anomalies might exist in certain structural proteins of the mutant which predispose them to greater lability at increased temperature of incubation. This may be the cause of the cellular disorganization observed microscopically and might also explain the partial if not complete remission which ensues on addition of pantothenate and/or amino acids and sodium chloride. It is pertinent that Kuraishi(8) has observed that the production rate of dead cells due to pantothenate deficiency is decreased in the presence of 0.2 M sodium chloride or 0.1 M sodium sulfate.

Summary. Data were presented which revealed that the paucity of growth of a mutant strain of yeast at 38°C is not only related to an inability to carry out the biosynthesis of pantothenic acid but also the requirement for an increased osmotic pressure or perhaps simply a high solute concentration. Results were discussed in terms of possible mechanisms by which these environmental conditions might serve to permit growth of the mutant at increased incubation temperatures. Since at lower temperatures the organism is genetically capable of synthesizing pantothenic acid, the role of environment in genetic expression is reemphasized.

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L Phase Variants Related to Antibiotic Inhibition of Cell Wall Biosynthesis.* (28753)

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It is generally accepted that certain antimicrobial agents inhibit cell wall biosynthesis. Biochemical demonstration of this action by penicillin and D-cycloserine has been reported by Strominger(1,2) who has measured the accumulation of cell wall precursors in different bacteria exposed to these antibiotics. Parallel studies have shown that bacitracin, novobiocin, and gentian violet also interfere with cell wall synthesis(1). Jordan(3) has demonstrated the inhibition of cell wall mucopeptide production by vancomycin. Biologic evidence for a similar action can be obtained by observing protoplast formation and/or L phase growth when selected bacteria are exposed to antimicrobial agents under appropriate environmental conditions. If L phase growth occurs, it has been assumed that only cell wall synthesis is interrupted. Penicillin and D-cycloserine are 2 antibiotics which are known to induce L phase growth,

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TABLE I. L Phase Variant Formation.

Organism	Penicillin		D-cycloserine†		Bacitracin		Novobiocin		Gentian violet		Vancomycin	
	LPC*	LB*	LPC	LB	LPC	LB	LPC	LB	LPC	LB	LPC	LB
Staphylococcus, 236	+	+	+	+	0	0	0	0	0	0	0	0
239	+	+	—	—	0	0	0	0	0	0	0	0
7632	+	+	—	—	0	0	0	0	0	0	0	0
13	+	+	+	+	0	0	0	0	0	0	0	0
Proteus rettgeri 52	+	+	+	+	0	0	0	0	0	0	0	0
Clay	+	+	+	+	0	0	0	0	0	0	0	0
Proteus vulgaris 18	+	+	+	+	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa	+	+	+	+	0	0	0	0	0	0	0	0

* LPC = L phase colonies. LB = Large bodies. + present, 0 absent, — not examined
† Reference(4).

thus confirming the biochemical studies for a primary action on cell wall formation(4).

We have studied the ability of bacitracin, novobiocin, gentian violet and vancomycin to induce L phase growth in selected bacteria to determine by a simple biologic experiment whether their only or primary action is interference with cell wall biosynthesis.

Materials and methods. Four staphylococcus strains (236, 239, 7632, 13), 2 *Proteus rettgeri* strains (52, Clay) a strain each of *Proteus vulgaris*(18) and *Pseudomonas aeruginosa* were transferred from tryptic digest broth to agar plates (Oxoid sensitivity test medium or trypticase soy medium (BBL)) containing 3% sodium chloride and 20% inactivated human ascitic fluid, heated at 56°C for 30 minutes. The plates were heavily streaked with the test organisms. Penicillin (125,500 μg/ml), bacitracin (12,500 μg/ml), novobiocin (100,000 μg/ml), gentian violet (100,000 μg/ml), and vancomycin (100,000 μg/ml) were added in wells cut into the agar. The plates were incubated semianaerobically at 37°C by the Fortner method(5). They were examined for L phase growth after 4 and 7 days by the stained cover slip agar block method(6). L phase variants were not reported unless typical L colony growth was observed with a "fried egg" appearance and large body formation.

Proteus rettgeri 52 was transferred from tryptic digest broth to PPLO broth (Difco) with 20% ascitic fluid and either 1.5% or 3% sodium chloride. After 6 hours of incubation at 37°C, serial dilutions of penicillin, bacitracin, novobiocin and vancomycin were added to the cultures. Examination by phase microscopy for protoplast formation was carried out 16 hours later. Protoplast formation was reported when large amounts of clearly spherical, enlarged forms of bacteria were seen.

Results. All 8 strains of bacteria examined demonstrated L phase growth in plates to which penicillin had been added. Six of these organisms had previously been shown to produce L phase variants when exposed to D-cycloserine(4). None of the strains exhibited L growth when exposed to bacitracin, novobiocin, gentian violet or vancomycin. Although some swollen forms were seen, there were no typical L colonies produced and no large body formation was visible (Table I).

Examination of *Proteus rettgeri* 52 in broth demonstrated protoplast formation upon exposure to penicillin as well as to bacitracin, novobiocin and vancomycin. Varying concentrations of the respective antibiotics provided what was ostensibly optimum conditions for protoplast formation (Table II).

Discussion. If one assumes that the primary or only site of action of an antimicrobial agent is upon cell wall synthesis, then one may also assume that L phase variants will be produced when there is biochemical evidence of interference with cell wall synthesis. Such is the case with penicillin and D-cycloserine.

The inability to produce L phase variants with bacitracin, novobiocin, gentian violet and vancomycin in organisms which are

TABLE II. Protoplast Formation.

Penicillin			Bacitracin			Novobiocin			Vancomycin		
	NaCl			NaCl			NaCl			NaCl	
μg/ml	1.5%	3.0%	μg/ml	1.5%	3.0%	μg/ml	1.5%	3.0%	μg/ml	1.5%	3.0%
620	+	+	20,000	0	0	1,000	0	0	1,000	±	+
310	+	+	10,000	0	0	500	0	0	500	+	+
155	+	±	5,000	0	0	250	0	0	250	+	0
78	+	0	2,500	0	0	125	0	0	125	±	0
39	+	0	1,250	+	0	62	+	+	62	0	0
20	±	±	625	0	0	31	0	±	31	0	0
10	±	0	312	+	+	16	0	0	16	0	0
5	±	0	156	0	0	8	+	0	8	0	0
2	±	0	78	0	+	4	0	0	4	0	0
1	0	0	39	0	0	2	0	0	2	0	0

+ many protoplasts, ± few protoplasts, 0 no protoplasts

known to be L forming bacteria, casts doubt upon the hypothesis that these agents act only or primarily by interference with cell wall synthesis. The production of large spherical organisms, *i.e.*, protoplasts, does give evidence that the cell wall is attacked. The appearance of these forms is presumably attributable to interference with cell wall biosynthesis. However, if this were the only site of action, one would expect not only protoplast formation but actual L phase growth with typical colonies and large bodies visible after 4 to 7 days. This L growth would be such that it could be maintained in serial transfer.

It therefore follows that the antimicrobial agents investigated, namely, bacitracin, novobiocin, gentian violet and vancomycin cannot act solely by interfering with cell wall synthesis. They must exert additional actions elsewhere upon other metabolic or reproductive pathways.

Summary. The effect of bacitracin, novobiocin, gentian violet and vancomycin upon organisms known to be L forming bacteria was studied. L phase growth could not be produced although protoplast formation occurred in liquid media. It is concluded that these agents cannot act solely by interference with cell wall biosynthesis.

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Chemical Protection Against Lethal Effects of Nitrogen Mustard in Rats.* (28754)

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Nitrogen mustard (HN2) is routinely dissolved in physiological saline solution before injection. Commercial saline, in its turn, often

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