TABLE II. Influence of Porcine Gamma Globulin and Bovine Serum Solids Administered by Different Procedures on Blood Serum Gamma Globulin Content of One-Day-Old Pigs. (Each value represents % of total protein as gamma globulin for an individual animal.)

Controls	Porcine gamma globulin			Bovine serum solids	
	Orally			Orally	
Cows' milk	Orally	with trypsin inhibitor	Parenterally (i.p.)	Orally	with trypsin inhibitor
0	0	0	35	9	7
0	6	9	29	0	0
0	0	5	44	0	0
0	0	0	40	0	5
	9		27	0	
	0		41	2	
	0		37	8	
			31	6	
			16	0	
				3	

traperitoneally but not when administered orally it is evident that processes related to the alimentary tract prohibited gamma globulin from reaching the blood stream. This indicates that sows' colostrum contains a component(s) that enables the gamma globulin to be absorbed from the alimentary tract without being digested. Evidence accumulated in this work indicates that trypsin inhibitor alone is not responsible and that the component(s) is not present in lyophilized serum solids in effective amounts. The failure of cows' colostrum to give a response with baby pigs suggests a species difference in the colostrum of the pig and the cow or in factors affecting the absorption of gamma globulin by the pig and the calf.

Summary. Data have been presented which show that porcine gamma globulin, porcine and bovine serum solids and cows' colostrum do not cause a marked or consistent response in the serum gamma globulin level of one-day-old pigs when administered orally. However, parenteral administration of porcine gamma globulin gave a response which approached that obtained with nursing pigs. The inclusion of trypsin inhibitor with orally administered porcine gamma globulin and bovine serum solids did not affect the serum gamma globulin level.

1. Laskowski, M., Jr., and Laskowski, M., *J. Biol. Chem.*, 1951, v190, 563.

2. Carle, B. N., and Dewhirst, Wm. H., *J. Am. Vet. Med. Assn.*, 1942, v101, 495.

3. Foster, Joseph F., Friedell, Robert W., Catron, Damon and Dieckmann, Merwin R., *Arch. Biochem.*, 1951, v31, 104.

Received August 31, 1954. P.S.E.B.M., 1954, v87.

L Colonies from Hemolytic Streptococci: New Technic in the Study of L Forms of Bacteria.*† (21297)

JOHN T. SHARP.‡ (Introduced by Louis Dienes.)

From the Department of Medicine and Bacteriology, the Massachusetts General Hospital and the Robert W. Lovett Memorial Foundation, Harvard Medical School, Boston, Mass.

The peculiar, small, fragile growth form usually designated as an L form has been isolated previously from alpha hemolytic streptococci(1). L forms from this species were obtained using technics successful in other species, in that of a large number of strains exposed to penicillin on horse serum agar plates, a few yielded L type colonies. Using these methods L type colonies have not been obtained from beta hemolytic streptococci, although many attempts have been made in this laboratory in the past several years, and during the past winter alone we have examined between 50 and 100 strains.

Results. Positive results have been obtained

* The expenses of this investigation have been defrayed in part by grants from the Public Health Service, National Institute of Arthritis and Metabolic Diseases and the Commonwealth Fund.

† This is publication No. 170 of the Robert W. Lovett Memorial Foundation for the study of crippling disease.

‡ Trainee, National Institute of Arthritis and Metabolic Diseases.

only after much experimentation with various media. The decisive factor has proved to be very simple, namely the concentration of electrolyte. Thus of 5 strains of group A streptococci that have been tested repeatedly on .25 M to 1.1 M NaCl plates, all have repeatedly produced L type colonies, although with varied regularity. Of 21 other strains of beta hemolytic streptococci, most of which are group A, that have been tested a single occasion on .5 to .58 M NaCl plates, 5 strains have produced L colonies. The optimal concentration of salt has not been determined but L type colonies have been observed in one strain with .25 M NaCl and in several strains with 1.1 M NaCl; transition to L forms has not been observed to occur at concentrations of NaCl below this, when other electrolytes were present in low concentrations, and NaCl has not been tested in higher concentrations. Several other salts have also been found to favor the development of the L colonies, including KCl, NH_4Cl, $CaCl_2$, $MgCl_2$, and Na_2HPO_4. Wide ranges in concentration of these electrolytes have not been studied, but L colonies have been observed on plates containing as little as .18 M of the bivalent cations and .14 M Na_2HPO_4.

Increased salt concentration favors the production of L forms not only from the streptococci but also from other species. This has been observed with the colon bacillus. Although scanty L growth of *E. coli* has been observed previously(2), a sufficiently abundant growth of these L forms has been obtained to permit successful subculture of the L forms for the first time by increasing the salt concentration of an otherwise appropriate medium.

Another indication of the importance of the salt concentration in the cultivation of L forms has been observed with a strain of alpha streptococci that has been carried in this laboratory for more than a year as a known L former. With this strain it has been possible to devise a medium in which all elements are chemically defined except for horse serum and agar. With this system it has been found that the concentration of electrolyte is critical for the successful transformation to the L form, but the required concentration is of a much lower order than with the beta streptococci, and is within the range usually employed in bacteriological media.

The technic with which L forms were isolated from beta hemolytic streptococci is as follows. The appropriate concentration of electrolyte was incorporated in plates containing a tryptic digest of beef heart infusion as base, 1.3% agar, and 10% horse serum inactivated at 56°C for 30 minutes. The plates were heavily inoculated with an 18-24 hour bacterial growth and about 1000 units of penicillin was deposited in a small trough cut in the agar. The plates were incubated at 36°C anaerobically, using the Fortner biological method. After a few days L type colonies appeared. These developed most regularly at the margin of the area where bacterial growth was inhibited.

The transition into L forms occurred in the same way as in other species. Some of the cocci increased greatly in size producing round or polygonal forms of 10-20 μ. The L growth started from these. (See photographs.) With .08-.16 M NaCl exposure to penicillin irregularly produces some pleomorphism with round forms 3-5 μ occurring but without further development. With .25 M NaCl one of 3 strains examined produced many large bodies and infrequent L colonies. Although observations are yet limited, transition into the L form has not been observed in the absence of anaerobic incubation, but growth on subculture is satisfactory with aerobic incubation.

L colonies develop on the high salt plates with high concentrations of penicillin (1000 U/cc or more) incorporated in the media but growth is less regular and less abundant than with the technic described above. After transition to the L form has occurred growth on subculture is not influenced by high concentrations of penicillin in the media.

The L forms isolated from the group A streptococci are similar both in the gross appearance of the colonies and in the microscopic morphology to the L forms of the alpha streptococci and present in every respect the characteristic properties of L forms. The original strains of hemolytic streptococci have been regained from the L forms of 2 strains. This was accomplished on

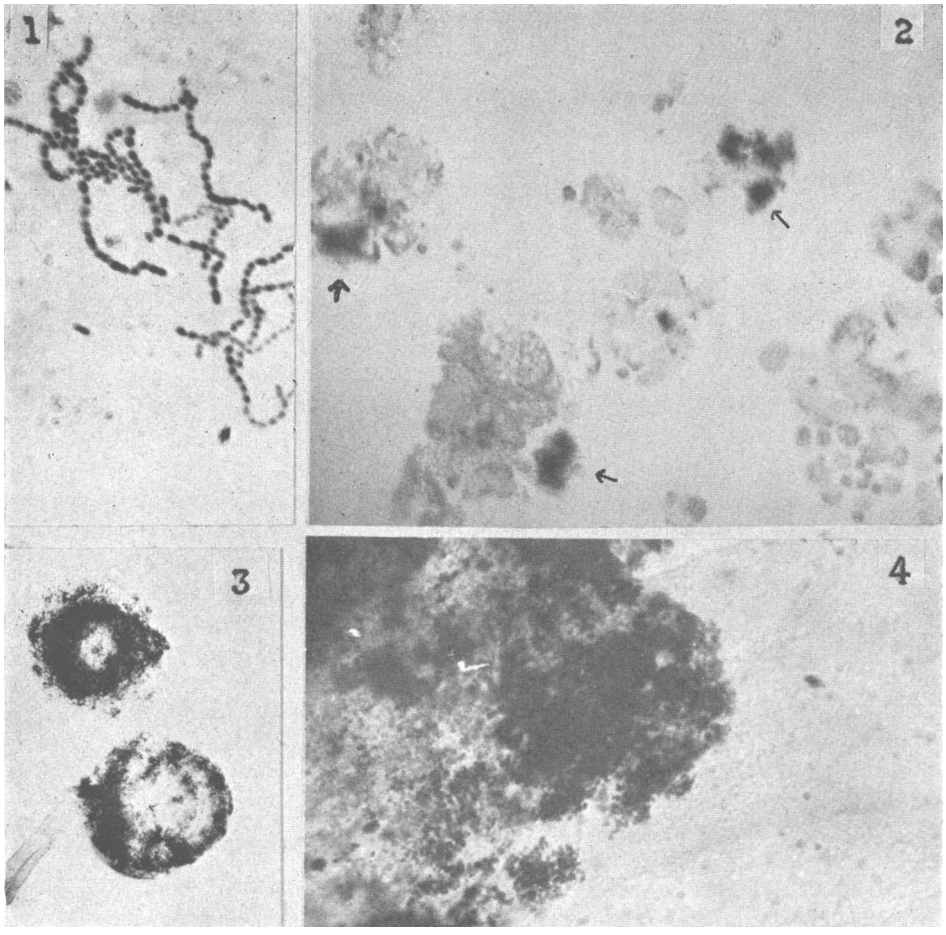

FIG. 1. Group A hemolytic streptococcus grown on plain agar, magnified 900×.

FIG. 2. Group A streptococcus exposed to penicillin on salt medium. The cocci are swollen to large bodies and several small L colonies (marked with arrows) are developing. Magnification 100×.

FIG. 3. Large L colonies of group A streptococcus magnified 100×.

FIG. 4. The edge of an L colony magnified 900×. The spreading growth is into the agar.

(Photographs 1 and 2 were made from wet preparations; 3 and 4 from dried preparations.)

.58 M NaCl plates containing no penicillin, the plates being incubated aerobically. The inocula were from the fifth serial transplants of the L form on plates containing high concentrations of penicillin. The serological properties of the L forms of the hemolytic streptococci have not been studied.

Discussion. L forms were isolated rarely, from exceptional strains of a few species, before the effectiveness of penicillin to produce such forms was noticed. However, a great many strains could not be induced to produce L forms with the use of penicillin even though isolation of L colonies from other strains indicated that the particular species was capable of producing such forms. It is reasonable to expect that the technic described in this note—*i.e.*, the combined use of penicillin and the appropriate electrolyte concentration—will materially extend the number of positive results. If this expectation is correct this technic will also provide an opportunity for more conclusive study of the biological and pathological significance of the L forms.

The isolation of L forms from group A streptococci is especially interesting because of the obscure nature of the pathogenic role of the streptococcus in certain diseases.

At the present time no hypothesis can be proposed to explain the influence of salts on the production of L forms.

Summary. L type colonies have been isolated from group A streptococci by the combined effect of penicillin and concentrations of various salts considerably higher than usually incorporated in bacteriological media. The appearance and morphology of these cultures correspond to those isolated from the alpha streptococci. There is reason to believe that the technic described will be helpful in the isolation of L forms of other bacterial species.

1. Dienes, L., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 579.
2. ———, *ibid.*, 1939, v42, 636.

Received August 31, 1954. P.S.E.B.M., 1954, v87.

Dietary Conditions Affecting Oxidation of Tyrosine by Rat Liver, with Particular Reference to Thiamine.* (21298)

JEAN P. KRING AND J. N. WILLIAMS, JR.
From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison, Wis.

The oxidation of tyrosine by mammalian liver enzymes proceeds through several steps before the end-products of the reaction, fumaric and acetoacetic acids, are obtained (1-6). Considerable work has been carried out to determine what cofactors are required for the various steps(5-11). Pyridoxal phosphate, ascorbic acid, and glutathione have been shown to be involved in the oxidation of tyrosine. Williams and Sreenivasan(9-11) found that the dye, 2,6-dichlorophenolindophenol (DCPP) could stimulate this oxidation in addition to the stimulation afforded by glutathione and ascorbic acid. It was considered possible that the DCPP in low concentrations might act as a substitute for a labile cofactor of an unknown nature. Boiled extracts of the active enzyme preparation also produced a stimulation of tyrosine oxidation by dialyzed or aged enzyme preparations in addition to the stimulation produced by glutathione and ascorbic acid.

Upon examination of the possible scheme for tyrosine oxidation, it becomes evident that the oxidation of 2,5-dihydroxyphenylpyruvic acid to homogentisic acid involves an oxidative decarboxylation. In view of the well known participation of thiamine-containing coenzymes in oxidative decarboxylation reactions, it was of interest to investigate the oxidation of tyrosine by enzyme preparations from livers of thiamine deficient rats as compared to those from normal control animals. If thiamine was active in promoting oxidation of tyrosine, its possible identity with the labile cofactor of the DCPP-substituting factor suggested by Williams and Sreenivasan(9-11) might be established.

Experimental. Since thiamine is active in the form of thiamine pyrophosphate (TPP) or, in certain systems, as lipothiamide pyrophosphate (LTPP)†(12), these substances were added to enzyme preparations obtained from normal and thiamine deficient rats. Dialyzed enzyme preparations, both from normal and thiamine deficient animals, have also been employed. A combination of a thiamine deficiency and dialysis of the enzyme preparations would be expected to remove thiamine-containing cofactors more completely than either treatment alone. Male rats of the Sprague-Dawley strain weight 90-110 g were divided into two groups. One group was fed a complete purified ration, and the other the same ration with thiamine omitted. The rations consisted of 20% vitamin-free casein,

* Published with the approval of the Director of the Wisc. Agric. Exp. Station. Supported in part by contract between Regents of the University of Wisconsin and the Office of Naval Research, Washington, D. C., and the Nutrition Foundation, N. Y.

† We wish to thank Dr. Lester J. Reed of the University of Texas, Austin, for the samples of LTPP.