cells (selectively, but orthochromatically, with neutral red and acridine yellow, metachromatically with Unna's mixture and toluidine blue). Some material with similar staining properties was also found in the blood of the adrenal venules; here it usually adhered to erythrocytes.

A comparison with typical mast-cells was then made by staining sections of the thymus of the same animals on the same slide with their adrenals. It was found that the ordinary mast-cells of the connective tissue in the thymus are smaller, their shape is more regularly ovoid and they contain much smaller and more intensely staining granules than the medullary mastocytoid elements.

Discussion. On the basis of the evidence available to us, it is impossible to advance any hypothesis concerning the possible histogenesis or function of the mastocytoid cells. It should be pointed out however that, after treatment with ACTH-preparations without added carboxymethylcellulose, we have not observed any such mastocytoid elements in the medulla, nor metachromatic granulations in the cortical cells. On the other hand, control experiments with the carboxymethylcellulose-containing solvent alone, gave positive results. Presumably, the mastocytoid elements in the adrenal medulla are due to ingestion by histiocytes of metachromatically staining carboxymethylcellulose granules.

Summary. Observations on rats indicate that treatment with large doses of an ACTH-preparation, to which carboxymethylcellulose was added as a retarding agent, results in the development of mast-cell-like elements within the adrenal medulla. At the same time, in some cortical cells and in adrenal-vein blood, occasional granules appear, which give the characteristic staining reactions of mast-cell granules. Carboxymethylcellulose - solutions containing no ACTH produce similar changes.

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Received October 24, 1955. P.S.E.B.M., 1955, v90.

Further Observations on L Forms of *Alpha*-hemolytic Streptococci.*† (22133)

W. HIJMANS‡ AND L. DIENES.

Departments of Bacteriology and Medicine, Mass. General Hospital, and Robert W. Lovett Memorial Foundation for Study of Crippling Disease, Harvard Medical School, Boston, Mass.

Isolation of L-type cultures from 2 strains of *alpha*-hemolytic streptococci was described in 1953(1). Many attempts at isolation of these forms from *alpha*-hemolytic streptococci in previous years had been unsuccessful. This paper contains the description of two methods which permit with some regularity the finding of strains of *alpha*-hemolytic streptococci which produce L forms. Data are also presented concerning the occurrence of such strains in humans and observations on a pleomorphic strain of *alpha*-hemolytic streptococcus which produced L forms spontaneously without the influence of penicillin.

After the characteristic appearance of L colonies of *alpha*-hemolytic streptococci and the conditions under which they grow became

* The expenses of this investigation have been defrayed in part by grants from the Commonwealth Fund and the U. S. Public Health Service.

† This is publication No. 188 of the Robert W. Lovett Memorial Foundation for the Study of Crippling Disease.

‡ Clinical and Research Fellow in Medicine, Mass. General Hospital, and Research Fellow, Harvard Medical School, under a stipend from Netherlands Organization for Pure Research (Z.W.O.). Present address: Department of Rheumatology, University Hospital, Leyden, Netherlands.

known, specimens from the human mouth, urethra and vagina were cultivated on appropriate plates. Bacterial growth was inhibited in a part of the plate by penicillin. In cultures made from the mouth, colonies of pleuropneumonia-like organisms usually developed on the inhibited areas and among these occasionally were found much larger colonies which corresponded in appearance to L-type colonies of streptococci. In 3 out of approximately 80 cultures examined, it was possible to subculture these large L-type colonies. They were first subcultured on penicillin plates. When the cultures were allowed to age in the absence of penicillin, *alpha*-hemolytic streptococci appeared in all. Each of these streptococcus cultures produced L-type growth on exposure to penicillin and evidently originated from the reversion of the L forms. The development of L-type colonies corresponding to those of streptococci was not observed in cultures made from the urethra or vagina although streptococcus colonies often developed abundantly on the plates.

These observations were made on the usual horse serum medium containing 0.5% NaCl. After it was observed that on plates containing a high concentration of NaCl or some other electrolytes many *beta*-hemolytic streptococci produced L forms(2,3) it seemed of interest to examine the behavior of *alpha*-hemolytic streptococci on such media.

Preliminary experiments were made by planting tooth scrapings and throat swabs on horse serum agar containing 3% NaCl and exposing part of each plate to penicillin. The development of L colonies of streptococci was observed only rarely on these plates. In a more systematic study made with throat swabs, a mixture of many colonies of *alpha*-hemolytic streptococci from a blood agar plate was inoculated on another plate to check the purity of the culture. From this culture large inocula were transferred to broth and the test plates were then heavily seeded with this broth culture. In this way each test plate received an inoculum originating from a number of colonies found in the same specimen. This procedure was adopted because trials with the descendants of single colonies had remained negative. An increased NaCl content of the media only rarely favored the development of L-type colonies. However, an increase in the phosphate content either of the sodium or potassium salt, had a marked effect. Abundant development of L-type colonies was observed with concentrations of phosphate between 0.2 and 0.4 M. Only one strain gave a slight growth of L forms with 0.1 M. Concentrations higher than 0.4 M were not tested. Ten out of 40 of such mixed cultures produced L forms on the phosphate plates. Two of the 40 produced L-type colonies with high concentrations of NaCl also, but less abundantly than with the phosphate. The influence of the phosphates was not due to differences in pH as the pH was the same in the chloride and phosphate plates. None of these 40 cultures produced L-type colonies without an increased salt concentration.

Subculture of these L forms was successful only on plates with an increased phosphate content indicating that the high concentration of phosphate ions was important for their growth. The transformation of the cocci into the L forms was probably induced by the penicillin, just as on the regular media. In the case of the strains of *alpha*-hemolytic streptococci which produced L-type colonies on the usual horse serum media, a high phosphate concentration in the medium neither enhanced the transformation of such strains into L forms nor was necessary for the subculture of the L forms.

On the basis of these observations the following procedure can be recommended to obtain L-type cultures of *alpha*-hemolytic streptococci. Plates are made using a tryptic digest of beef heart or beef heart infusion as base, 1.3% agar, and 10% horse serum inactivated at 56°C for 30 minutes. Three ml of a mixture of 20% Na_2HPO_4 and 1.4% KH_2PO_4 is added to 15 ml of the medium. The plates are heavily inoculated as described above. The plates are incubated for 2 hours to allow multiplication of the cocci and then about 1000 units of penicillin are deposited in a small trough cut in the agar. The plates are incubated at 36°C for 4 to 5 days under anaerobic conditions using the Fortner biological method. The behavior of the cultures was consistent. Six strains which originally

produced L forms continued to produce such forms in 4 to 13 subcultures when the transfers were made with heavy inocula. Three to five subcultures of seven strains, which originally did not produce L forms, did not yield this type of growth. The use of an agar base of different composition did not increase the percentage of positive strains.

The observations described indicate that approximately one out of 4 humans harbors in his mouth *alpha*-hemolytic streptococci which, under the conditions employed, produce L-type colonies. Further study has shown that only a few of the single colonies in the throat cultures of such persons will produce L colonies. One throat culture known to be positive was studied more thoroughly and only the descendants of 1 out of 20 single colonies gave positive results. Further subcultures of these 20 colonies behaved similarly. The descendants of negative colonies were always negative. Subcultures obtained by transferring large inocula from a positive strain were always positive. However, only about one-fifth of the descendants of isolated colonies remained positive. This was followed through 4 generations. These observations indicate that not all but only a certain percentage of the cocci in a positive colony transmit to their descendants the ability to produce L forms under the conditions of these experiments. A similar observation was made previously in this laboratory with a Gram positive bacillus(4).

An interesting strain of *alpha*-hemolytic streptococcus was isolated on several occasions from the blood of a patient in the weeks preceding his death.§ The source of the septicemia was an old kidney abscess; no endocardial lesions were found at autopsy. The organisms were extremely pleomorphic both in the original broth flasks and in transplants on blood agar plates without penicillin. Normal cocci were not observed and the cultures consisted of large round bodies, small granules and long filaments, some with swellings. The cultures resembled those of *Streptobacillus moniliformis*. The nature of the organisms was established only after several transfers, when the usual coccal form of growth was resumed. When transferred immediately after isolation to horse blood and horse serum agar plates without penicillin and without increased electrolyte concentration, L colonies developed spontaneously among the pleomorphic coccus colonies. When the cultures were exposed to penicillin only L colonies developed.

Discussion. The L forms of bacteria can be recognized at present only by the growth of characteristic colonies. This criterion was used in all cases reported in this paper. The occurrence of large bodies or granules in the cultures, as accepted by several authors,‖ is not sufficient proof of transformation of bacteria into L forms. Such morphological elements are often present in cultures without signs of multiplication. The expressions "L forms," "L-type growth" and "L cultures" are synonymous and refer to the same fundamental phenomena.

The L-type cultures described in this report undoubtedly originated from *alpha*-hemolytic streptococci. The macroscopical and microscopical appearance of the cultures is characteristic, and differs from that of PPLO which could conceivably contaminate the cultures. The serological specificity of the L forms studied earlier and in the course of the present studies was similar to that of the parent streptococci. The observation that streptococci reappeared in the cultures of L forms and that these cocci reproduced L forms under appropriate conditions is further evidence for the relation between the cocci and these L forms.

The tendency of strains of *alpha*-hemolytic streptococci to produce L forms is as variable as in the other species. The behavior of these strains, as far as the production of L forms is concerned, is in many respects similar to that observed in *Bacteroides*(5). One strain of *alpha*-hemolytic streptococcus produced L forms spontaneously on the usual media. A few strains produced L forms on similar

§ We are indebted to Lawrence J. Kunz, bacteriologist of the Massachusetts General Hospital, for this strain and many others mentioned in this publication.

‖ See discussion in (3).

media under the influence of penicillin. High concentrations of NaCl increased slightly and high concentrations of phosphate increased markedly the number of strains which produced L forms. The behavior of the descendants of individual coccal chains of a strain which produced L forms was variable. In some strains the majority of the cocci present in a single colony lost the ability to produce L forms.

It is of interest that in several species such as *Bacteroides* and the hemophylic bacteria, which are the usual inhabitants of the human body, pleomorphism and spontaneous production of L forms is more frequent among the strains isolated from pathological processes than among those isolated from their usual habitat. Similarly, the strain of *alpha*-hemolytic streptococcus which produced L forms spontaneously originated from an abscess of long duration, and was characterized by extreme pleomorphism. This relation cannot be due entirely to the influence of antibiotics, because some of these observations were made before they came into use.

Summary. High concentrations of potassium and sodium phosphates incorporated in the media markedly increased the number of strains of *alpha*-hemolytic streptococci which produced L-type growth when exposed to penicillin. High concentrations of NaCl produced this effect only slightly. Using the phosphate media, strains of *alpha*-hemolytic streptococci which produced L forms were found in about one-fourth of the throat cultures examined. One unusual strain of *alpha*-hemolytic streptococcus produced L forms spontaneously without exposure to penicillin.

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Received October 25, 1955. P.S.E.B.M., 1955, v90.

Endogenous Formation of Hippuric Acid.* (22134)

MARVIN D. ARMSTRONG, FU-CHUAN CHAO, VIRGIL J. PARKER, AND PATRICIA E. WALL.
(Introduced by Emil L. Smith.)

Laboratory for Study of Hereditary and Metabolic Disorders, and Departments of Biological Chemistry and Medicine, University of Utah College of Medicine, Salt Lake City.

Occurrence of hippuric acid in mammalian urine has been known since its isolation by Liebig(1) in 1829. It has usually been supposed to arise by conjugation of benzoic acid of dietary origin with glycine in the body, and a great part of biochemical investigations of hippuric acid formation have been concerned with glycine metabolism. Recently, presence of hippuric acid in urine of fasting humans was reported and a possible endogenous origin was suggested for it(2). Shortly thereafter, Schreier *et al.*(3) observed a dilution in specific activity of radioactive benzoic acid in fasting rats, and suggested that an endogenous formation of benzoic acid could explain their results. In addition, they cited other observations, unsupported by experimental data, which were in agreement with this idea. Our investigation indicates that in humans and in the white rat there is a small amount of hippuric acid produced endogenously and that in the rat the aromatic ring of phenylalanine can, to a slight extent, serve as a precursor for the benzoic acid moiety. A major proportion of hippuric acid present in urine of humans and of rats receiving a natural diet is, however, derived from dietary precursors.

* This research was supported by grants from the National Institutes of Health, U. S. Public Health Service.

Methods. Albino rats (Sprague-Dawley), weighing 300 to 400 g, were fed either Purina