AMINO SUGARS IN L FORMS OF BACTERIA AND PLEUROPNEUMONIA-LIKE ORGANISMS

JOHN T. SHARP

Department of Medicine, Harvard Medical School, and Massachusetts General Hospital, Boston, Massachusetts

Received for publication 29 April 1963

Abstract

Sharp, John T. (Harvard Medical School, Boston, Mass.). Amino sugars in L forms of bacteria and pleuropneumonia-like organisms. J. Bacteriol. 86:692–701. 1963.—Studies of several bacterial L forms and their parent bacteria have revealed similar amino sugars in the two forms of microorganisms, with the exception that muramic acid was absent from the streptococcal and staphylococcal L forms. In contrast, muramic acid was found in a Proteus L form. Some of the properties of the muramic acid component in the Proteus L form are described. The solubility of this material was observed to be very different in the L form as compared with the bacterium. Some implications of this observation are discussed. Two strains of pleuropneumonia-like organisms did not contain muramic acid.

A variety of features characterize pleuropneumonia-like organisms (PPLO) and L forms of bacteria, and tend to distinguish them from ordinary bacteria. Unusual nutritional requirements make PPLO and L forms among the most fastidious microbial agents grown on artificial media. These organisms are also universally resistant to penicillin in very large amounts, frequently not being inhibited by as much as 10,000 to 40,000 units of penicillin per ml of culture media, and this resistance is not due to penicillinase. The minimal reproductive unit is small, probably in the neighborhood of 0.2 to 0.3 μ in size. When grown on artificial media, both PPLO and L forms of bacteria tend to imbode into the agar, a phenomenon almost never seen with bacteria. Many L forms and some PPLO show a marked preference for growth on a solid medium rather than in liquid. Along with the difficulty of finding appropriate conditions for their cultivation, we infer that many of these organisms grown under the best of circumstances must have a considerably longer generation time than do bacteria. Finally, one of the most characteristic features of this group of agents is the unusual degree of fragility and pliability of all elements of PPLO and L form cultures. Although comments regarding their unusual plasticity predate Nowak’s (1929) report, his graphic description of the elements in a culture of PPLO as appearing like thick liquid protoplasm emphasized this property.

More recently, Dienes and Weinberger (1951) commented that the L form of Proteus appeared to be the growth of the bacterium without its usual hard outer membrane, or what would today be called the cell wall. These early morphological observations were confirmed by immunological studies, when it was demonstrated that the group-specific polysaccharide was absent from the L forms of group A streptococci (Sharpe, Hjimans, and Dienes, 1957). Considered in the light of McCarty’s (1952) earlier demonstration that the group-specific polysaccharide was a significant component of the bacterial cell wall and did not occur in other structural components, the observation that the streptococcal L form lacked this material provided clear-cut chemical and immunological evidence of a major structural alteration occurring in the process of transformation to the L form.

Attention was then turned to the Proteus L
form, and it was demonstrated that somatic antigen was present in the L form to a significant extent, but reduced in amount when compared with the bacterial form (Sharp and Dienes, 1959). To determine the significance of this observation, bacterial organisms were fragmented and separated into cell-wall and cytoplasmic fractions by differential centrifugation. Approximately 50% of the total somatic antigen was found in the cytoplasm and about 50% in the cell wall. Since it was not clear whether the occurrence of such a large amount of somatic antigen in the bacterial cytoplasm represented fragmentation of the cell wall during preparation, the occurrence of soluble antigen within the cytoplasm, or the presence of somatic antigen in a structure closely related to both the cell wall and cytoplasm, such as a cytoplasmic membrane, the observation of significant amounts of somatic antigen in the bacterial L forms did not allow inferences as to the type of chemical alteration associated with transformation to the L form.

Studies were then directed to a survey of amino sugars in bacteria and in bacterial L forms. Salton (1953) and Cummins and Harris (1956) demonstrated that amino sugars were concentrated to a significant extent in bacterial cell walls. Strange and Powell (1954) discovered the unusual amino sugar, muramic acid, and this material was demonstrated to be a structural component of the cell wall. Muramic acid also was found in a nucleotide fraction (Park and Strominger, 1957) and was shown to increase in amount when the bacteria were inhibited by penicillin (Park and Johnson, 1949). These observations established the importance of amino sugars in the bacterial cell wall and suggested that these materials were in some way related to the mechanism of action of penicillin. A study of amino sugars in L forms was undertaken because it seemed likely to provide further information as to the nature of the alteration which we identify as the transformation of the bacterium to its L form.

**Materials and Methods**

Strains of organisms studied included: Proteus vulgaris strain 18 and its L form, obtained from R. Tulasne, University of Strasbourg; Streptococcus pyogenes strain GL8 (group A, type 19), obtained from J. Seal, U.S. Naval Medical Research Unit 4, and its L form; Staphylococcus aureus strain H, obtained from J. T. Park, Tufts University, School of Medicine, Boston, Mass., and its L form; and mycoplasmataceae (PPLO) strains Campo, obtained from L. Dienes, Massachusetts General Hospital, Boston, and K5, obtained from H. E. Adler, School of Veterinary Medicine, University of California, Davis.

Bacterial forms of *P. vulgaris* and *S. aureus* were grown with aeration in either nutrient broth or Field's (1956) broth. Aeration was carried out with a sponge aerator or with a platform shaker, and growth was continued for 16 to 20 hr. Streptococci were cultivated in Field's (1956) broth or Todd-Hewitt broth (Difco) in stationary cultures. L forms of these organisms were grown in the same media used for growth of the bacteria with the addition of 5% horse serum or ascitic fluid. Sodium chloride (3%) was added to the liquid media for growth of staphylococcal L forms and streptococcal L forms. *Proteus* L forms were grown in liquid culture in Fernbach flasks with a thin layer of broth in each flask, thus providing a large surface area to take advantage of the film of surface growth this organism produced. After a film had begun to form, the flasks were shaken to sink the original surface layer; this was continued as long as further films developed, usually for a period of 5 to 7 days. PPLO were grown in Field's (1956) broth with 5% horse serum or ascitic fluid added.

Bacteria or L forms after full growth in appropriate media were collected by centrifugation and washed once with an ice-cold sodium chloride solution of a concentration identical to that employed in the liquid growth medium. The organisms were then extracted rapidly with ice-cold 0.6 N trichloroacetic acid by mixing thoroughly in a glass tissue grinder in a cold room and centrifuging immediately. A second extraction was carried out in most instances, with a small volume of trichloroacetic acid. The extract was then dialyzed, with two or three changes of 0.15 M sodium chloride in most instances as well as several changes of distilled water. In the few instances when dialysis was carried out with distilled water initially, special note will be made in the following text. The extracts after dialysis were lyophilized or concentrated on a vacuum still.

The residue remaining after extraction with trichloroacetic acid was washed twice with ether.
to remove any residual trichloroacetic acid, suspended in a small volume of water, and lyophilized.

Appropriate samples of the residue or the extract were hydrolyzed with 6 N HCl for 6 hr in sealed tubes in a boiling-water bath. The hydrolysate was dried over P₂O₅ in vacuo and used for further studies. When column chromatography was performed, the hydrolysate from as much as 330 mg of extract or residue was applied in 0.3 M HCl to a Dowex-50 (H⁺) column (1 by 33 cm). (For larger amounts, a 2 by 21.5 cm column was used.) Elution of amino sugars from Dowex was carried out with 0.3 M HCl (Gardell, 1953). Fractions were collected (1 or 3 ml, depending on which column size was used). After a flow of 200 to 250 ml for the small column and 600 to 700 ml for the large column, 4 N HCl was used to elute remaining amino sugars. Amino sugar was measured on a sample from every second or third fraction by a modified Elson and Morgan procedure (Immers and Vasseur, 1950).

Paper chromatography was performed by a descending technique for unidimensional runs. The solvents are noted in the Results. Ninhydrin degradation in some instances was carried out on paper by the two-dimensional technique (Stoffyn and Jeanloz, 1954). In other instances, ninhydrin degradation was carried out in sealed capillary tubes, and the product was applied to paper for unidimensional development (Stoffyn and Jeanloz, 1954).

Amino sugars were acetylated for chromatographic identification by the procedure outlined by Roseman and Daffner (1956).

**RESULTS**

*P. vulgaris bacterial form.* Glucosamine, galactosamine, and muramic acid were recovered from *P. vulgaris* (bacterial form) and identified. In addition, a fourth component reacting in the Elson and Morgan procedure was eluted with 4 N HCl but not identified. Glucosamine and galactosamine were found in both the acid-soluble extract and the insoluble residue. Muramic acid was found only in the residue, as was the component eluted with 4 N HCl. Figure 1a is an elution diagram showing the results of column chromatography on the hydrolysate obtained from 332 mg of the acid-insoluble residue of the *Proteus* bacteria. The first distinct peak, A, appeared after approximately 40 ml of effluent. This material was thought to be incompletely hydrolyzed material, because it did not move as rapidly as known monosaccharides and amino sugars on paper chromatography.

After approximately 90 ml of eluting fluid were collected, a sharp peak, B, appeared which was glucosamine. On the descending limb of this second peak, the color product in the Elson and Morgan reaction had a greater optical density at 510 than at 530 m, in two collections. This is shown by the points identified by dots in region C. After this, a third peak, D, appeared at approxi-
Of these or talosamine. degraded to amine or inose, Jeanloz (1959).

The ninhydrin with amino sugars obtained from the bacterial material was degraded to fructosamine after treatment in HCl, but its position in the elution diagram suggested that it could be fructosamine or a diaminoo sugar recently described by Sharon and Jeanloz (1959).

Figure 2 is a paper chromatogram on material obtained from the bacterial form of *P. vulgaris*. Tube 104, although not from the column illustrated in Fig. 1a, is comparable to peak B, and tube 130 is similar to peak D. In Fig. 2, known amino sugars and test materials were degraded with ninhydrin in sealed capillary tubes, and the product was run by single-dimension chromatography. The material in peak B degraded to arabino, and was thus identified as either glucosamine or mannosamine. The material in peak D degraded to lyxose, and was either galactosamine or talosamine. Figure 3 established the identity of these materials. Peak B from the column shown in Fig. 1a was acetylated and chromatographed on borate-treated paper (Cabib, Leloir, and Cardini, 1953). The test material had the same *Rf* as did two control substances: (i) a commercial sample of acetylglucosamine, and (ii) a sample of glucosamine acetylated simultaneously with the peak B material. The peak D substance on acetylation ran at the same *Rf* as did galactosamine acetylated at the same time as the peak D material. These studies established that *P. vulgaris* contains glucosamine and galactosamine.

The identity of the material on the down slope of peak B was further studied by pooling this region, called C, evaporating the hydrochloric acid, and placing the residue on Amberlite IR 120 buffered at pH 6.05. The material not retained by the resin formed a color product in the Elson and Morgan reaction with a stronger absorption at 510 than at 530 m, a characteristic of muramic acid (Strange and Dark, 1956). When paper chromatography was performed, the material ran similarly to muramic acid.

The acid-soluble extract of *P. vulgaris* 18 bac-
acteria, after thorough dialysis, was hydrolyzed and analyzed by the same procedures detailed above for study of the residue. Only glucosamine and galactosamine were identified.

*P. vulgaris* 18 L form. Figure 1b is the elution diagram obtained from an experiment in which 750 mg of acid-insoluble residue of the *P. vulgaris* 18 L form were hydrolyzed and put on a Dowex-50 (H⁺) column. Glucosamine and galactosamine were recovered and identified by paper chromatography with ninhydrin degradation and acetylation (Fig. 4 and 5). The component eluted by 4 N HCl from columns loaded with bacterial hydrolysate was found in the L-form residue. However, no suggestion of color reversal occurred in large amounts of material from the descending slope of peak B or the early ascending portion of peak D. When material from the C region was placed on Amberlite IR 120, the eluate gave no reaction in the Elson and Morgan procedure.

Figure 1c presents the elution diagram from the acid-soluble, nondialyzable extract of *P. vulgaris* 18 L. In this instance, before peak B began to descend, another large peak with color reversal appeared. The material in this peak was pooled and placed on an Amberlite IR 120 column. An amount of 273 μg of nonabsorbed material which showed color reversal was recovered. (This represented approximately 0.0135% when related to the dry weight of residue from which this extract was obtained.) Chromatography on paper with two different solvent systems showed this material to migrate with an RF similar to muramic acid (Fig. 6 and 7). Peak D from this column was identified as galactosamine by ninhydrin degradation (Fig. 8).

These studies demonstrated glucosamine and galactosamine in the acid-insoluble residue of the *Proteus* L form. Muramic acid and galactosamine were identified in the acid-soluble extract, and glucosamine was probably present, although attempts at identification were not made. The component eluted by 4 N HCl from the column loaded with bacterial residue was also present in the L-form residue.

Other experiments designed to recover mu-
ramic acid from Proteus L forms are included in Table 1. On several occasions, a significant quantity of material reacting in the Elson and Morgan procedure was obtained from acid-soluble extracts. In those instances in which the extracts were dialyzed against sodium chloride solutions, the material recovered as muramic acid varied between 0.0135 and 0.0200% of the dry weight of original organism or of residue. In these instances, the recovered material gave a color product with stronger absorption at 510 than at 530 mµ.

Similar studies on Proteus bacteria gave an estimate of muramic acid content varying between 0.0815 and 0.131%. Although it should be emphasized that these techniques were not quantitative, the magnitude of difference between the L form and bacteria is believed to indicate a significant difference.

Further studies on the acid-soluble nondialyzable extract of the Proteus L form are shown in Table 2. In one experiment, attempts to characterize the material showed that a significant proportion of the extract was soluble in 80% ethanol. A higher proportion of muramic acid than of total hexosamine was recovered from the ethanol-soluble fraction, and a moderate amount of labile phosphorus was present in this fraction. Calculated as micromoles, there was some similarity between the quantity of muramic acid
and of labile phosphorus. In another experiment in which the acid-soluble extract was treated with 90% ethanol, absorption spectra in ultraviolet wavelengths from 240 to 280 μm were determined. The acid-soluble, ethanol-soluble portion had a definite peak at 260 μm, whereas the ethanol-insoluble fraction had none (Table 3).

**Other L forms and PPLO.** After observations on the *Proteus* L form indicated that an acid-soluble fraction contained muramic acid, streptococcal and staphylococcal L forms were studied in a similar fashion (Table 4). Material showing color reversal in the Elson and Morgan procedure and an E' on Dowex-50 (H⁺) between 1.03 and 1.06 (E' of glucosamine equals 1) appeared in the acid-insoluble residue of bacterial forms of both streptococci and staphylococci. A similar material was also found in the acid-soluble extract of the staphylococcus. No such material was found in either L form, and it was concluded that these L forms did not contain muramic acid. Two PPLO strains were studied, and no evidence of muramic acid was found in these.

<table>
<thead>
<tr>
<th>Table 1. Amino sugar recovered from Amberlite IR 120 (pH 6.05) columns</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organism</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em> 18, bacterium</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><em>P. vulgaris</em> 18, L form</td>
</tr>
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<td></td>
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<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>L form</td>
</tr>
<tr>
<td>Campo PPLO</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

- All material eluted from Amberlite IR 120 (pH 6.05) columns with water giving reaction in modified Elson and Morgan procedure was calculated as muramic acid, whether identified as such or not. Per cent was related to dry weight of organism, if that figure was available. In all other instances, per cent was related to dry weight of the trichloroacetic acid residue.
- Muramic acid has been reported to give a color product with greater optical density at 510 than at 530 μm. Glucosamine gives a product with greater absorption at 530 μm.
- Paper chromatography results similar to muramic acid in one solvent system.
- Trichloroacetic acid residue.
- Trichloroacetic acid-soluble extracts: 1, dialyzed against water; 2, 3, and 4, dialyzed against NaCl and water. Extract 2 was a total amino sugar; extract 4 was applied directly to Amberlite. Extract 3 gave results similar to muramic acid in two solvent systems.
- Trichloroacetic acid-soluble extract; dialyzed against NaCl and water.
- Applied directly to Amberlite.
of muramic acid was found in the eluate. Figures
given are estimates of the maximal amount pos-
sibly present.

**Discussion**

The studies reported here show that muramic
acid is absent from staphylococcal and strepto-
coccal L forms, and from the Campo PPLO (a
human genital strain) and the K1 PPLO (a goat
strain). In contrast, the Proteus L form was ob-
erved to contain this unusual amino sugar. It
was of particular interest that the solubility of
the muramic acid component was strikingly
different in the L form as compared with the
bacterial form. Because muramic acid is present
in some bacteria in a considerable amount as an
acid-soluble nucleotide fraction, particularly
when the bacteria are inhibited by penicillin,
studies were undertaken to establish whether
the acid-soluble fraction in the Proteus L form
containing muramic acid was a nucleotide. The
ultraviolet absorption spectrum of that portion
of the acid extract soluble in 80% ethanol and
the occurrence of acid-labile phosphate in this
fraction suggested that this material was a nu-
cleotide. However, retention of this material,
in spite of prolonged dialysis against sodium chloride
solutions, would appear to indicate that any
nucleotide present was either sufficiently poly-
merized to prevent dialysis or conjugated to
another molecule of considerable size. Whether
the muramic acid is a portion of such a molecule
was not established.

The occurrence of muramic acid in one L form
and its absence in others is of interest. Several
possible explanations deserve consideration. In
the first instance, the transformation of a bac-
terium to its L form may not involve the same
biochemical step in all cases. Thus, it is con-
ceivable that the chemical alteration may involve
total loss of the cell wall in the staphylococcus
and the streptococcus on transformation to an L
form, but in Proteus there may be a loss of mate-
rials other than those containing muramic acid.
In addition, type 3A and 3B L forms of Proteus
might well vary in regard to their biochemical
alteration. An alternative consideration is that
the muramic acid in this Proteus L form was not
incorporated into a structural component of the
cell but was present in the cytoplasm. In either
case, it is clear that penicillin does not inhibit

**Table 2. Studies on nondialyzable trichloroacetic
acid extract from L form of Proteus vulgaris 18**

<table>
<thead>
<tr>
<th>Component</th>
<th>Fraction soluble in 80% ethanol</th>
<th>Insoluble fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg</td>
<td>µg</td>
</tr>
<tr>
<td>Total hexosamine</td>
<td>363.8</td>
<td>493.2</td>
</tr>
<tr>
<td>&quot;Muramic acid&quot;</td>
<td>262.4</td>
<td>144.0</td>
</tr>
<tr>
<td>Labile phosphorus</td>
<td>26.7</td>
<td>48.0</td>
</tr>
<tr>
<td>(1.05 µM)</td>
<td>(0.85 µM)</td>
<td></td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>84.6</td>
<td>124.0</td>
</tr>
</tbody>
</table>

* A 27-g (wet weight) amount of Proteus vulgaris
18 L form was extracted in ice 0.6 N trichloro-
acetic acid. Residue was 1.944 g (dry weight).
After dialysis against 0.15 m NaCl and water suc-
cessively, dry weight of extract was 23.7 mg; 13.9
mg of this extract were soluble in 80% ethanol.

**Table 3. Absorption spectrum of ethanol-soluble
and ethanol-insoluble fractions of nondialyzable
trichloroacetic acid extract from L form
of Proteus vulgaris 18**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Wavelength (µm)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>240</td>
</tr>
<tr>
<td>Ethanol soluble*</td>
<td>0.209</td>
</tr>
<tr>
<td>Ethanol insoluble</td>
<td>0.380</td>
</tr>
</tbody>
</table>

* A 27.8-g (wet weight) amount of Proteus vul-
garis 18 L form was extracted with ice 0.6 N tri-
chloroacetic acid. The extract was dialyzed
against 0.15 m NaCl and then against water. Dry
weight of the extract was 23.0 mg; 13.3 mg were
soluble in 80% ethanol. For optical density deter-
minations, the fractions were dissolved in 1 N
HCl. The soluble fraction was in a concentration
of 144 µg/ml; the insoluble fraction, 62.5 µg/ml.
† Results are expressed as optical density at the
indicated wavelengths.
TABLE 4. Summary of identification data on amino sugars of L forms and PPLO recovered from Dowex-50 (H⁺) columns

<table>
<thead>
<tr>
<th>Organism</th>
<th>Fraction</th>
<th>Glucosamine</th>
<th>Muramic acid</th>
<th>Galactosamine</th>
<th>4 N Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ninhydrin</td>
<td>Acetyl</td>
<td>R_P</td>
<td>Ninhydrin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>degradation</td>
<td>glactation</td>
<td>530:310 Ratio</td>
<td>degradation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IR 120</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Paper</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>chromatography</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>Extract</td>
<td>+</td>
<td>1.03</td>
<td>&lt;1.0</td>
<td>1.20</td>
</tr>
<tr>
<td>group A, GL8</td>
<td>Residue</td>
<td>+</td>
<td>1.18</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>S. pyogenes L form</td>
<td>Extract</td>
<td>+</td>
<td>1.05</td>
<td>&lt;1.0</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td>Residue</td>
<td>+</td>
<td>1.06</td>
<td>&lt;1.0</td>
<td>1.21</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Extract</td>
<td>+</td>
<td>1.05</td>
<td>&lt;1.0</td>
<td>1.15</td>
</tr>
<tr>
<td>H</td>
<td>Residue</td>
<td>+</td>
<td>1.06</td>
<td>&lt;1.0</td>
<td>1.17</td>
</tr>
<tr>
<td>S. aureus L form</td>
<td>Extract</td>
<td>+</td>
<td>1.05</td>
<td>&lt;1.0</td>
<td>1.19</td>
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<tr>
<td></td>
<td>Residue</td>
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<td>1.06</td>
<td>&lt;1.0</td>
<td>+</td>
</tr>
<tr>
<td>Proteus vulgaris 18,</td>
<td>Extract</td>
<td>+</td>
<td>1.05</td>
<td>&lt;1.0</td>
<td>+</td>
</tr>
<tr>
<td>bacterium</td>
<td>Residue</td>
<td>+</td>
<td>1.06</td>
<td>&lt;1.0</td>
<td>+</td>
</tr>
<tr>
<td>P. vulgaris L form</td>
<td>Extract</td>
<td>+</td>
<td>1.05</td>
<td>&lt;1.0</td>
<td>+</td>
</tr>
<tr>
<td>Campo PPLO</td>
<td>Whole org</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>K₁ PPLO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a If material was obtained from an organism that degraded with ninhydrin to arabinose as determined by paper chromatography, + appears in the hexosamine column. If material degraded to lyxose, a + appears in the galactosamine column.

b If the acetylation product was similar to acetylgalactosamine or acetylgalactosamine by paper chromatography, a + appears in the appropriate column. A blank space indicates test was not performed.

c R_P glam = R_P relative to glucosamine.

d Ratio 530:310 refers to optical density of color product formed in Elson and Morgan reaction.

e A + appears in this column if material was not retained on Amberlite IR 120 buffered to pH 6.05.

f A 4 N compound refers to material found in peak E in several column chromatograms.

g Triehloroacetic acid-soluble extract.

h Triehloroacetic acid-insoluble residue.

i An unidentified peak with R_P relative to glucosamine of 1.35 to 1.48 was observed in the hydrolysate of this strain.

molecule sufficiently large to be nondialyzable. Additional studies to determine whether this component in the Proteus L form is a portion of the membrane structure would be of considerable interest. The presence of muramic acid in a soluble component within the cytoplasm would be entirely compatible with the thesis proposed by Park and Strominger (1957) and Strominger (1962) that incorporation of the muramic acid-peptide complex into the cellular structure is blocked by penicillin. On the other hand, if muramic acid were found in the Proteus L membrane, this would constitute strong evidence that some chemical alteration other than prevention of incorporation of the muramic acid component was responsible for the altered morphology of the Proteus L form.

ACKNOWLEDGMENTS

This study was supported in part by a grant from the Commonwealth Fund, New York, N.Y., and by grant A-1250 from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service.

The author gratefully acknowledges the stimulus and advice of Louis Diener.

The author wishes to express appreciation for the technical assistance of Tsun-Ye Kwan.

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