places, either during isolation or on fractionation, to give fragments of different molecular weight. The merits of these two possible explanations will be discussed.

Although the transforming activity is associated largely with different chromatographic fractions of DNA in the two prototrophic strains used, the ratio of transforming frequencies for the three markers studied are the same.

P. H. acknowledges the award of a Research Studentship by the Medical Research Council. The authors thank Dr M. S. Kelly for the gift of cultures.

Ayad, S. R., Barker, G. R. & Weigold, J. (1968). *Biochem. J.* **107**, 387.
Mandell, J. D. & Hershey, A. D. (1960). *Analyt. Biochem.* **1**, 66.
Sueoka, N. & Cheng, T. (1962). *J. molec. Biol.* **4**, 161.

The Cell Wall of a Chlorhexidine-Resistant *Pseudomonas*

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A strain of *Pseudomonas* capable of growth in media containing high concentrations of chlorhexidine had a lower capacity than normal strains for absorbing the bactericide from the growth medium (Bentley, Davies, Field & Roberts, 1968). This might be a reflection of an unusual wall structure. Bacteria were grown in Roux bottles at 30° in nutrient broth with 0·5% (w/v) glucose (150ml./bottle) with and without chlorhexidine digluconate (100μg./ml.) and were also grown on nutrient–agar plates from which they were scraped and suspended in 0·15M-NaCl. Cells grown without chlorhexidine were centrifuged and washed with cold 0·15M-NaCl; those grown with chlorhexidine were washed once with 0·1% (w/v) acetic acid and once in 0·1M-phosphate buffer, pH7·0, both at 2°. Cells were disrupted by shaking them with no. 12 Ballotini beads in a Mickle disintegrator for 15min. at 2°. Cell walls were isolated by centrifugation and resuspension five times in M-NaCl and five times in water, and then fractionated by a method based on that of Clarke, Gray & Reaveley (1967). Comparative analytical values for the walls of the chlorhexidine-resistant bacterium and for *Pseudomonas aeruginosa* N.C.T.C. 1999 (in parentheses) were: % of N 13·2, 11·1 (9·6, 8·9): % of P 0·62, 0·54 (1·8, 1·8): % of lipopolysaccharide (aqueous phase after phenol extraction of the wall) 5·8, 11·2 (22·7, 27·2); % of P in the lipopolysaccharide 1·76, 1·0 (4·9, 4·4). Although the values varied with different growth conditions (in each pair of values the first value is for liquid culture, the second for culture on agar) it was clear that the resistant *Pseudomonas* contained more nitrogen and considerably less phosphorus than *Ps. aeruginosa*. The greatest difference (eightfold) was in the amount of lipopolysaccharide phosphorus. The electrophoretic properties of these organisms differed: with *Ps. aeruginosa* 80% of the surface charge was due to a monobasic acid of pK about 4·5 and 20% to an acid of lower pK, whereas with the resistant organism the proportions were 94 and 6% respectively. Chlorhexidine readily precipitates nucleic acids from solutions and has far less effect on proteins (Hugo & Longworth, 1966; A. Davies, unpublished work); the resistant *Pseudomonas* may have acquired its resistance to chlorhexidine at least in part by a decrease in surface phosphate groups, thus decreasing the cell's ability to absorb the bactericide.

Bentley, M., Davies, A., Field, B. S. & Roberts, W. (1968). *Biochem. J.* **110**, 46P.
Clarke, K., Gray, G. W. & Reaveley, D. A. (1967). *Biochem. J.* **105**, 775.
Hugo, W. B. & Longworth, A. R. (1966). *J. Pharm., Lond.*, **18**, 569.

Intracellular Accumulation of *N*-Acetylhexosamines in *Micrococcus lysodeikticus*

By A. J. Garrett. (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

Deprivation of bivalent cations increases the intracellular concentration of a mucopeptide precursor in *Bacillus subtilis* W23 (Garrett, 1968). Mg^{2+} or Mn^{2+} are specific activators of all the mucopeptide-synthesizing systems isolated so far, and as the particulate enzymes from *Micrococcus lysodeikticus* have the highest requirement for Mg^{2+} (Anderson, Meadow, Haskin & Strominger, 1966), the effects of bivalent-cation starvation on cell suspensions of this organism were studied.

M. lysodeikticus 2665 cells from exponentially growing cultures were resuspended (1·5–2·0mg. dry wt./ml.) in 0·05M-phosphate buffer, pH7·0, containing DL-alanine, DL-glutamic acid, L-lysine, glycine (each 0·02%), glucose (1·0%) and chloramphenicol (0·005%). Incubation in this medium alone does not stimulate accumulation of *N*-acetylhexosamines, but EDTA (0·001M) caused a 10–15-fold increase in 1hr. Chromatography of the trichloroacetic acid-soluble material on Sephadex G-25 and on paper showed the presence of three nucleotide–*N*-acetylhexosamines. Two have been identified as the mucopeptide precursors, UDP-*N*-acetylmuramyl-Ala-Glu-Lys-Ala-Ala (44% of the hexosamine pool) and UDP-*N*-acetylglucosamine (32%). The third (24% of the pool) is a UDP derivative of an acidic hexosamine, with a uridine:

N-acetylhexosamine (measured as *N*-acetylglucosamine) ratio of 1·0:0·5 and a uridine:hexosamine (measured as glucosamine) ratio of 1·0:1·0. The hexosamine was obtained in maximum yield after hydrolysis of the nucleotide in 4N-HCl for 15min. at 100°; hydrolysis for longer times destroyed the sugar. On paper chromatograms the hexosamine behaved like the aminomannuronic acid (Perkins, 1963) from the cell-wall polysaccharide of *M. lysodeikticus*.

UDP-*N*-acetylaminomannuronic acid has been isolated recently by Rosenthal (1968). It is proposed, therefore, that deprivation of bivalent cations stimulates the accumulation of three cell-wall precursors in the cytoplasm of *M. lysodeikticus*. However, treatment of another coccus, *Staphylococcus aureus* H, with EDTA causes less than a twofold increase in its *N*-acetylhexosamine content.

It is noteworthy that abnormally high concentrations of UDP-*N*-acetylmuramyl-peptides can be induced by several different methods, but increased pools of UDP-*N*-acetylglucosamine have only been reported in *S. aureus* deprived of nitrogen (Ito & Saito, 1963), where only a transient accumulation occurred, and in *S. aureus* treated with 5-fluorouracil (Rogers & Perkins, 1960).

I thank Miss Shlomith Hampel for excellent technical assistance.

Anderson, J. S., Meadow, P. M., Haskin, M. A. & Strominger, J. L. (1966). *Arch. Biochem. Biophys.* **116**, 487.
Garrett, A. J. (1968). *Biochem. J.* **106**, 40P.
Ito, E. & Saito, M. (1963). *Biochim. biophys. Acta*, **78**, 237.
Perkins, H. R. (1963). *Biochem. J.* **86**, 475.
Rogers, H. J. & Perkins, H. R. (1960). *Biochem. J.* **77**, 448.
Rosenthal, S. (1968). *Abstr. 156th Meet. Amer. chem. Soc.* no. B35.

Formation of an *S*-Glucuronide by a UDP-Glucuronyltransferase and its Hydrolysis by β-Glucuronidase

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Thiol compounds can be conjugated with glucuronic acid in animals (see Marsh, 1966; Smith & Williams, 1966). One of these *S*-glucuronides, (*NN* - diethylthiocarbamoyl 1 - thio - β - D - gluco - pyranosid)/uronic acid, occurs in mammals given Antabuse, tetraethylthiuram disulphide (Kaslander, 1963; Strömme, 1965).

The various types of *O*-glucuronides are biosynthesized by glucuronyl transfer from UDP-glucuronic acid to the aglycone (see Dutton, 1966). This communication demonstrates formation of diethyldithiocarbamoyl *S*-glucuronide by the same route, and its hydrolysis by a β-glucuronidase preparation.

Diethyldithiocarbamate was incubated in mouse liver homogenate with UDP-glucuronic acid. In a modification of Kaslander's (1963, 1966) procedure, the incubation mixtures were acidified and extracted with butanol, the extracts were evaporated and chromatographed, and the chromatograms were sprayed with iodine–sodium azide reagent (Chargaff, Levine & Green, 1948). Two spots appeared, with R_F values corresponding to those of diethyldithiocarbamate and its *S*-glucuronide (Kaslander, 1963). On further chromatography, the latter spot behaved like authentic diethyldithiocarbamoyl *S*-glucuronide put through the same procedure. By the modified Tollens' test (see Dutton, 1966), hexuronic acid was shown to be present in this spot. No spot or hexuronic acid appeared when UDP-glucuronic acid was added after incubation or was incubated without substrate.

When UDP-[^{14}C]glucuronic acid was present during incubation, radioactivity coincided with this 'conjugate' spot. If the labelled 'conjugate' was incubated with preputial gland (Levvy, McAllan & Marsh, 1958) β-glucuronidase, all radioactivity left the conjugate and appeared in the position of free glucuronic acid; if glucarolactone (Levvy, 1952) was present with the β-glucuronidase, no release of radioactivity was observed. That the β-glucuronidase preparation hydrolysed *S*-β-glucuronides and that glucarolactone inhibited this hydrolysis was demonstrated with the authentic *S*-glucuronide; no information about the action of β-glucuronidase on *S*-glucuronides could be found in the literature.

The synthetic enzyme appears to be a UDP-glucuronyltransferase. No conjugate appeared when UDP-glucose, glucuronolactone or glucuronate replaced UDP-glucuronic acid during incubation. Some properties of this enzyme will be discussed.

We thank Dr J. Kaslander and Dr C. W. Pluijgers for generously giving us authentic *S*-glucuronide, Dr I. H. Stevenson for much radiochemical advice and facilities, and the Medical Research Council for a grant towards expenses.

Chargaff, E., Levine, C. & Green, C. (1948). *J. biol. Chem.* **175**, 67.
Dutton, G. J. (1966). In *Glucuronic Acid*, p. 186. Ed. by Dutton, G. J. New York: Academic Press Inc.
Kaslander, J. (1963). *Biochim. Biophys. Acta*, **71**, 730.
Kaslander, J. (1966). Ph.D. Thesis: University of Utrecht.
Levvy, G. A. (1952). *Biochem. J.* **52**, 464.
Levvy, G. A., McAllan, A. & Marsh, C. A. (1958). *Biochem. J.* **69**, 22.
Marsh, C. A. (1966). In *Glucuronic Acid*, p. 3. Ed. by Dutton, G. J. New York: Academic Press Inc.
Smith, R. L. & Williams, R. T. (1966). In *Glucuronic Acid*, p. 457. Ed. by Dutton, G. J. New York: Academic Press Inc.
Strömme, J. H. (1965). *Biochem. Pharmacol.*, **14**, 391.