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Carbohydrate Metabolism in *Pasteurella multocida*. III. Incorporation of Glucose Carbon into the Cell-Wall Polysaccharide.* (29002)

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A partially purified lipopolysaccharide antigen isolated by phenol extraction from *Past. septica (multocida)* was found to contain glucosamine, galactose and aldoheptose(1). This communication deals with the complete purification of the polysaccharide moiety of the antigen and its relationship to glucose metabolism.

Materials and methods were the same as used previously(2,3). Glucose-U-C^{14} (0.15 mC/mmole) was from the Radiochemical Centre, Amersham, Great Britain. Hexosamines were revealed in the chromatograms with the Elson-Morgan reaction(4). Paper electrophoresis was performed as described by Markham and Smith(5).

Results and discussion. Purification of polysaccharide. Step 1 (removal of capsular polysaccharide(6)). 9 g of acetone-dried *Past. multocida* (strain Beufort No. 28 from the Institute Pasteur, Paris) were shaken mechanically with 150 ml of 2.5% NaCl and 1 ml of toluene for 4 hours at 20°. The suspension was centrifuged, the supernatant poured off and the residue reextracted twice as above. The cells were suspended in 100 ml of boiling distilled water and shaken gently with glass beads for 2 minutes. After centrifugation at 12000 *g* the extraction was repeated with 50 ml of distilled water at 100° and the centrifugation was repeated. *Step 2* (extraction). The cell residue was suspended in 90 ml distilled water, warmed at 65° and treated with an equal volume of aqueous 90% (w/v) phenol solution at 65°(7,8). The mixture was stirred for 45 minutes at this temperature and then cooled rapidly at —2°. Upon centrifugation, the phenol phase was discarded. *Step 3* (acetone precipitation). The aqueous solution was poured into 10 vol of dry acetone at —10°, left for 24 hours at —20° and centrifuged. The sediment was washed 3 times with acetone at —10°, suspended in 20 ml distilled water, dialysed for 48 hours against running tap water and dried under vacuo. Yield: 708 mg. *Step 4* (removal of nucleic acid). An aqueous 1% (w/v) solution of polysaccharide was centrifuged twice at 100000 *g* for 3 hours. The sediment was dissolved in 60 ml distilled water and treated twice as above with cold acetone containing 0.3% of potassium acetate. The residue was dried under vacuo, redissolved in distilled water and centrifuged once at 100000 *g* for 3 hours. The pellet was dissolved in 10 ml distilled water, dialysed against distilled water for 48 hours at 4° and dried under vacuo. White colorless leaflets were obtained. Yield: 204 mg. Nucleic acid and protein content (determined spectrophotometrically(9)) were 0.2 and 0.6% respectively. *Step 5* (removal of lipid(10)). The polysaccharide was dissolved in 25 ml 0.01 N HCl at 100° for 45 minutes. The solution was cooled, centrifuged at 3000 rpm for 30 minutes, the pellet washed twice in the centrifuge with 0.01 N HCl and dried under vacuo. Yield: 134 mg. *Step 6* (ethanol precipitation). The polysaccharide was dissolved in

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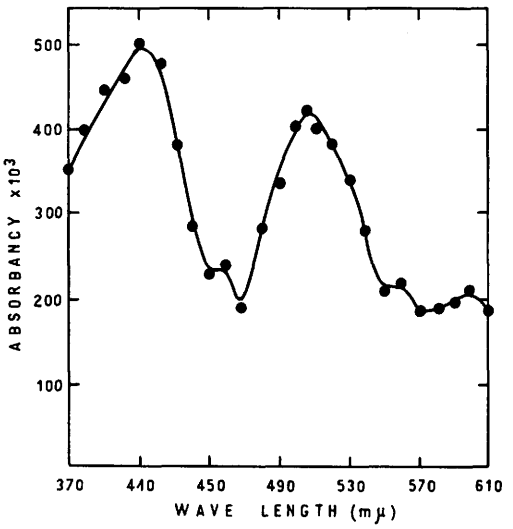

FIG. 1. Absorption spectrum of polysaccharide after treatment of 480 μg of material with the sulfuric acid-cysteine reaction(16). Readings were made at 24 hours in 1 cm cuvettes. The maximum at 505 mμ is due to aldoheptose, and that at 410 mμ to hexose.

water, 100 ml ethanol at 4° was added, and after 72 hours at 4°, the precipitate was centrifuged. Dissolution and precipitation were repeated twice. After drying under vacuo the yield was 40 mg. *Step 7* (electrophoresis). 5 mg of material was dissolved in 0.50 ml of 0.05 M borate buffer, pH 8.6, and subjected to electrophoresis at 20°, with 12 volt/cm (8 mA) on Whatman No. 1 paper washed previously with the same borate buffer. Several samples were run simultaneously for locating the spot and for analysis of the polysaccharide. After a 5 hour run, the paper was dried and sprayed with the aniline (11) or ninhydrin(12) reagents. The first revealed the location of the polysaccharide which migrated towards the anode whereas the ninhydrin reaction revealed the presence of 3 spots, one towards the cathode and 2 towards the anode, without overlapping with the polysaccharide. 43 mg of purified polysaccharide were heated with 6 ml N H_2SO_4 for 16 hours at 105° in a sealed tube. After cooling, the sulfate ions were removed with $Ba(OH)_2$, the hydrolysate concentrated under vacuo and chromatographed with the phenol-water or the butanol-propionic acid-water systems(13). Glucosamine was revealed with the Elson-Morgan and ninhydrin(12) reactions and glucose with the aniline reagent (11); both compounds were identified by cochromatography with pure specimens. Aldoheptose was identified by *a*) the R_f value in comparison with glucose R_f (4,14), *b*) the color reaction with *p*-anisidine(15) and *c*) the sulfuric acid-cysteine reaction (Fig. 1). The relationship of glucose metabolism(2) with the polysaccharide components is shown in Table I since the latter incorporated radiocarbon from glucose-U-C^{14} when this was used as substrate.

TABLE I. Incorporation of Glucose-U-C^{14} into the Polysaccharide of *Past. multocida*.

7.35 g of organism suspended in 60 ml mM phosphate buffer, pH 7.2, containing 6.4 mM of glucose-U-C^{14} (1.18 × 10^8 c.p.m.). After 60 min. incubation at 37° the cells were collected, and the polysaccharide extracted and purified (yield, 40 mg; sp. activity, 20 c.p.m./mg). After hydrolysis with NH_2SO_4 as described in text the digest was chromatographed bidimensionally with the phenol-water butanol-propionic acid-water systems(13), radioautographed and counted for C^{14}.

Distribution of C^{14} (%)		
Glucosamine	Glucose	Aldoheptose
50	24	19

Summary. The polysaccharide moiety of *Past. multocida* cell-wall antigen has been purified by precipitation with acetone and ethanol, ultracentrifugation, acid treatment and paper electrophoresis. The polysaccharide contains glucose, glucosamine and aldoheptose, which incorporated C^{14} after incubation of the bacteria with glucose-U-C^{14}.

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Role of the Appendix in Development of Immunologic Capacity.* (29003)

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Numerous experiments have shown that neonatal thymectomy in the mouse, rat, and hamster markedly reduces the immunologic capacity of the maturing animal(1-7). In rabbits, however, neonatal thymectomy has resulted in some depression of antibody production, but the differences between the antibody levels of the neonatally thymectomized animals and control groups have varied in magnitude and in statistical significance(8-10). Neither delayed hypersensitivity nor transplantation immunity has been discernibly affected by neonatal thymectomy in the rabbit, in contrast to the findings in other species(10,11).

In recent months evidence from a number of laboratories has suggested that immunologic development of the chicken is significantly affected by 2 lymphoid organs, the thymus and the bursa of Fabricius(12-16). Archer *et al*(17) have hypothesized that the rabbit appendix may be a homologue of the bursa of Fabricius of the chicken, based on derivation of both organs from epithelium of the gut, the similarity of their lymphoid development by a budding of follicles from epithelium, their striking morphologic resemblance at maturity, and the apparent status of the appendix as a thymus-independent organ. Thus, the limited effect of neonatal thymectomy on the lymphoid tissues and the immunologic response of the maturing rabbit might be a reflection of compensatory activity of the appendix.

The present studies provide the first experimental test of the hypothesis that the thymus and appendix function synergistically in the development of immunologic capacity.

Materials and methods. Litters of newborn New Zealand white rabbits were separated into groups for thymectomy-appendectomy, thymectomy-splenectomy, thymectomy, appendectomy, or sham-operation. The procedures were performed within 24 hours after birth. No litter was used if the doe had a gestation period of longer than 32 days. An effort was made to randomize the procedures within the litters, so that the number of each litter in each experimental group averaged about the same initially (thymectomy-splenectomy was not included as a procedure in all the litters, and this group is under-represented). The technique of thymectomy has been described(10). The animals were returned to their mothers in the animal quarters of the local rabbitry, and were maintained for 3 weeks on twice weekly doses of 0.2 ml penicillin (Crysticillin, Squibb, 300,000 units per ml). Thereafter, they were given a combination of tetracycline hydrochloride (Polyotic, American Cyanamid) and neomycin sulfate + methscopolamine bromide (Biosol-M, Upjohn) in their drinking

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