3580

On Carbohydrate-Like Specific Substance in the Colon Aerogenes Group.

JOSEPH TOMCSIK. (Introduced by Carl TenBroeck.)

From the Department of Pathology, Peking Union Medical College.

Specifically reacting polysaccharides have been isolated by various authors from pneumococci, pneumobacilli, tubercle bacilli and yeasts. Preliminary unpublished experiments convinced us that substances with varying chemical composition and carbohydrate content could be obtained easily and in large quantities from different encapsulated bacilli (*B. coli, B. anthracis* and encapsulated soil bacteria). *B. lactis aerogenes* was chosen in the present study, since in the early literature Emmerling[1] and Schardinger[2] succeeded in isolating a nitrogen free polysaccharide from the filtrated cultures of these organisms.

The strain chiefly used for the preparation of the polysaccharide was isolated from human feces. Its behavior in sugar media was typical, acid was produced on citrate agar, the indol reaction was negative and the Voges-Proskauer test positive. Capsule formation was distinct though not marked.

Following the method of Emmerling and Schardinger an attempt was made to isolate a carbohydrate from the filtrate of a synthetic fluid medium culture. The yield of specific substance was small and failed to cause precipitation in dilutions higher than 1:10,000 when layered over homologous immune serum. It was found, that even after 30 days incubation the bulk of the specific substance was associated with the bacterial bodies which still showed a small, but distinct capsule.

The method finally adopted was essentially the same as that described by Toeniessen.[3] At first the bacilli were cultivated on a synthetic medium solidified with agar containing ammonium phosphate as a source of nitrogen, and sodium citrate as the carbon source. Among many substances tried, the addition of small amounts of prophyl-red more than doubled the growth. In later work meat infusion agar was used with equally satisfactory results. The two days old growth from 100 Kolle flasks, suspended in distilled water and precipitated with alcohol, yielded 11 gm. of dried bacteria. This could be resuspended evenly in 1800 cc. distilled water. The capsules of the bacteria were found to be intact when the suspension was examined in Indian ink or stained with Hiss

capsule stain. After the addition of 200 cc. of 100 per cent KOH, the suspension was placed in the incubator (37° C.), and every half hour samples were taken out for the examination for capsules. These usually disappeared at the end of 3 or 4 hours and at this time the solution was separated from the bacteria by centrifuging. To the supernatant fluid acetic acid was added until no more precipitate (nucleoproteid) came down, after which the fluid was passed through a Seitz filter. Three times the volume of alcohol was added to the clear filtrate and 2.1 gm. white powder obtained. This powder was easily soluble in water or saline and gave specific precipitation with the corresponding immunserum when diluted 1:200,000. It contained 1.4 per cent nitrogen.

With this procedure 5 gm. material was collected for further purification. The alcohol precipitation was repeated six times in the presence of n/10 sodium acetate in slight alkalic reaction. The precipitin titer of the resulting powder was 1:300,000 (faint reaction in 1:1,000,000). The nitrogen determined both with Nesslerisation and according to the micro Kjeldahl procedure titrating with 0.005/n iodometric solutions was still 0.9 per cent. A 1:200 solution gave negative Biuret and Millon tests, was not precipitated after boiling with acetic acid or after the addition of trichloracetic acid, silver-nitrate, or Fehling solution. It gave partial precipitate with barium-sulphate and neutral lead acetate, and both the precipitate and the supernatant contained the specific substance. It was precipitated quantitatively by the addition of alkalic lead acetate, phosphotungstic acid and colloidal ferrihydroxide. With Benedict's reagent there was no reduction. After hydrolysis with n/H_2SO_4 (Optimum time 4 to 5 hours at 100° C.) its reducing substance counted as glucose corresponds to 66 per cent.

Further purification was attempted by absorption with aluminium-hydroxide. In this we followed the technic of Heidelberger, Goebel and Avery,[4] but instead of "A" grade aluminium hydroxide, we used "B" grade, which seemed to us easier to prepare and according to Willstätter highly effective in the purification of enzymes. Practically the same specific titer and nitrogen content was found after the absorption. Uranyl nitrate could not be used for further purification as it caused no precipitation.

Specific substances were prepared from five other strains of aerogenes with the same cultural and biological characteristics. None of these substances reacted specifically with the first immunserum. With one of these strains immunserum was produced in rabbits, which was precipitated by the homologous specific substance, in a dilution of 1:100,000 but again gave no reaction with the substances

obtained from the other strains. The strict specificity of these substances makes it difficult to attempt any typing by this means in such a heterologous group of organisms.

A similar substance isolated from encapsulated *B. coli* reacted also in very high dilution with homologous immune sera and showed the same narrow type specificity.

To determine whether these substances were antigenic, rabbits were injected in the same way as with the bacterial culture, but with considerably larger quantities of dry material. Precipitations were not produced.

Protein free hapten was isolated from a strain of *B. lactis aerogenes*. This substance consists chiefly of polysaccharides, but in spite of several attempts at further purification, its nitrogen content could not be reduced below 0.9 per cent. Similar substances obtained from several other aerogenes strains and from encapsulated *B. coli* showed a strict individual specificity, no cross reactions were observed.

---

[1] Emmerling, O., *Ber. d.d. chem. Ges.*, 1900, xxxiii, 2477.

[2] Schardinger, F., *Cent. f. Bakt.*, Abt. II, 1902, viii, 144.

[3] Toeniessen, E., *Cent. f. Bakt.*, Orig., 1920-21, lxxxv, 225.

[4] Heidelberger, M., Goebel, W. F., Avery, O. T., *J. Exp. Med.*, 1925, xlii, 701.

3581

Anaphylactic Shock Produced by a Soluble Specific Substance Largely Carbohydrate in Nature.

JOSEPH TOMCSIK. (Introduced by Carl TenBroeck.)

From the Department of Pathology, Peking Union Medical College.

In order to study the relation of the precipitin test to anaphylaxis, and to determine whether a substance largely carbohydrate in nature and failing to give protein reactions would produce shock, the following experiments were made. The aerogenes specific substance described in the previous paper, containing 0.9 per cent N and giving a precipitate with immune serum when diluted 1:500,000, was used for the tests.

Twenty guinea pigs were sensitized by the intraperitoneal injection of 1 cc. aerogenes immune serum, and the response of their uteri tested, using the Schultz-Dale method. The uteri of all these animals reacted to the specific substance when tested from 2 hours to