Paperelectrophoretic Studies on Material from Encapsulated *Bacillus anthracis*

B. K. NORDBERG and W. THORSELL

Department of Bacteriology and Department of Chemistry, The Royal Veterinary College of Sweden, Stockholm, Sweden

The earlier presumption of a complex composition of the capsule from *Bacillus anthracis* by Nordberg and Thorsell¹ seems to be confirmed in these paper-electrophoretic studies combined with different dyeing methods.

Materials and methods. *In vivo* encapsulated *B. anthracis* microbes were isolated from rabbits according to the method of Smith *et al.*² About 0.5 ml of the microbes were suspended in 25 ml of dist. water and left at 20° C until the somata were freed from their capsules. This procedure was followed by microscopic examination of smears stained with Löffler's methylene blue according to Heim³. The cell bodies retained their normal staining properties. The suspension obtained was centrifuged and the supernatant was evaporated in vacuum at 30° C to a volume of about 0.5 ml to form the "capsular material".

Hemolyzed rabbit blood was prepared by diluting normal rabbit blood with one volume of dist. water. The substances isolated from *B. anthracis in vivo* (poly-D-glutamic acid and polysaccharide) by Smith and Zwartou⁴ were used as 1 % w/v in dist. water.

These materials were used in equivalent amounts for paper electrophoretic studies at pH 5.3 (0.1 M acetate buffer-solution), pH 6.5 (0.1 M phosphate buffer-solution) and pH 8.6 (veronal-acetate buffer-solution according to Michaelis).

After drying at 105° C for half an hour the papers were dyed with

1) chlorine dioxide-benzidine according to Reindal and Hoppe⁵.

2) Amidoblack 10 B according to Grassmann and Hannig⁶ with the modification described in "Type LKB 3276 Paper Electrophoresis Equipment. Instructions for use" (AB. LKB, Sweden).

3) periodic acid-fuchsin sulphurous acid according to Wunderly and Piller⁷.

4) Löffler's methylene blue, using a modification of the method described by Strange and Harkness⁸, whereby the paperstrips were washed twice in 80 % ethanol, treated with

Symbols: 1 = Chlorine-dioxide-benzidine. 2 = Amidoblack 10 B. 3 = Periodic acid-fuchsin-sulphurous acid. 4 = Löffler's methylene blue. a = *B. anthracis* "capsular material". b = Hemolyzed rabbit blood. c = *B. anthracis* poly-D-glutamic acid. d = *B. anthracis* polysaccharide. S = Starting line.

0.02 M hydrochloric acid in 90 % ethanol, washed in ethanol, dried and placed in methylene blue solution for 20 minutes.

Results at pH 5.3 are shown in Fig. 1. The broader lines indicate a relatively greater staining intensity.

Discussion. It appears from Fig. 1 that *B. anthracis* "capsular material" contains an anodically rapidly migrating fraction stainable by the chlorine dioxide-benzidine method in the same way as the poly-D-glutamic acid isolated from *B. anthracis* (1 a, 1 c). Other *B. anthracis* components stainable by chlorine dioxide-benzidine or amidoblack in the "capsular material", perhaps, for example, the very weakly staining fraction seen in 2 a, are difficult to recognize as they, due to interferences, may be concealed by material from hemolyzed blood (1 a, 1 b; 2 a, 2 b).

The attempt to demonstrate other capsular components in the "capsular material" through the use of periodic acid - fuchsin sulphurous acid and methylene blue stain-

ing methods show that the only fraction of the "capsular material" stainable with methylene blue is the highly negatively charged one (4 a). This seems to indicate the occurrence of *B. anthracis* poly-D-glutamic acid (4 c). The more widespread area of the "capsular material" fraction could be due to various interferences (cf. 1 a, 2 a), at least one of which Cromartie *et al.*⁹ observed in their electrophoretic investigations on material from anthrax oedema and which is perhaps visualized in the very weakly staining component of the "capsular material" (2 a). The slowly migrating fractions of the "capsular material" show a fuchsin-stainable component (3 a) almost at identical position with isolated *B. anthracis* polysaccharide (3 d). As the "capsular material" used seems to contain real capsular material and very little or no somatic material and as the other fuchsin-stainable components from hemolyzed blood (3 b) are not visible (3 a) this could mean that the carbohydrate component proposed for the *B. anthracis* capsule is identical with or similar to the isolated *B. anthracis* polysaccharide.

We want to express our sincere gratitude to Prof. H. Hedström, Prof. K. Sjöberg and Doc.

H. Svensson, Stockholm, Sweden, for their interest and valuable help, and to Dr. H. Smith, Porton, England, for his kind gift of *B. anthracis* poly-D-glutamic acid and polysaccharide.

1. Nordberg, B. K. and Thorsell, W. *J. Bacteriol.* **69** (1955) 367.
2. Smith, H., Keppie, J. and Stanley, J. L. *Brit. J. Exptl. Pathol.* **34** (1953) 471.
3. Heim, L. *Münch. med. Wochschr.* **51** (1904) 426.
4. Smith, H. and Zwartou, H. *Biochem. J. London* **56** (1954) viii.
5. Reindal, F. and Hoppe, W. *Naturwiss.* **40** (1953) 221.
6. Grassman, W. and Hannig, K. *Hoppe-Seyler's Z. physiol. Chem.* **290** (1952) 1.
7. Wunderly, C. and Piller, S. *Klin. Wochschr.* **32** (1954) 425.
8. Strange, R. E. and Harkness, N. *Nature* **171** (1953) 77.
9. Cromartie, W. J., Bloom, W. L., Watson, D. W., Heckly, R. J., Kegeles, G., Freed M., McGhee, W. J., Weissman, N. and Graf, L. H. *J. Infectious Diseases* **80** (1947) 1, 121.

Received August 8, 1955.