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## Cl. Botulinum Type D Sp. N.

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During the past year the laboratory has received several specimens of botulinus-like organisms through the courtesy of Drs. P. J. du Toit and E. M. Robinson, Onderstepoort, Pretoria, South Africa. Although these cultures differ in many ways from those isolated in this country, one strain secured from lamziekte carrion deserves special consideration.

The fluid portion of the original culture was toxic for mice. The antitoxic sera Type A, B and C failed to protect the animals against this poison irrespective of the fact that the symptoms and lesions were in every respect typical for botulism. A highly potent toxin was obtained in beef heart-peptic digest mash (1 cc. = 20,000 guinea pig M.L.D.) and Hibler's brain medium. Protection experiments with the known type antitoxins and a serum supplied by Dr. E. M. Robinson known as "*Cl. parobotulinum bovis antiserum*" were made on guinea pigs.

The results clearly indicate that the toxin of one of the South African strains of *Cl. botulinum* is different from either *Cl. botulinum* Type B and C or *Cl. parobotulinum* Type A and B.

In pure culture the organism resembles the anaerobe described by Theiler and Robinson.<sup>1</sup> It is a long, slender, Gram-positive, unusually pleomorphic rod which is actively motile under strictly anaerobic conditions. In egg-medium (Rettger) numerous subterminal closteridium-like spores are formed, while in beef heart and other media spores are rarely observed. Rhizoid, non-hemolytic surface colonies appear on properly prepared glucose blood plates. Growth is good and a small amount of gas is formed in beef heart, egg and milk media provided a vaseline seal is used. The proteolytic properties are low (Sørensen's figures 1st day 0.5, 14th day 4.0), about the same as the non-ovolytic Type B and slightly higher than the Type C strains.

The cultures have a strong odor of butyric acid. Indol is not produced. Glucose, levulose, sucrose, lactose, maltose, glycerine,

<sup>1</sup> Theiler and Robinson, *Z. f. Infektionskr. d. Haustiere*, 1927, xxxi, 165; 11th and 12th Reports of Director of Veterinary Education and Research, Dept. Agri., Union of S. Africa, 1927, II, 1099.

inosite and galactose are fermented when the test medium is composed of 0.3% Liebig's extract, 0.5% Difco peptone and 1% carbohydrate. No gas is liberated, but the change in pH is marked. In many of the sugars a final pH after 4 days incubation is 4.6 to 5.0. *Cl. botulinum* Type D is the only strain thus far encountered which fails to produce gas from carbohydrate. It also differs from other strains in its action on sucrose and lactose. Toxic and non-toxic variants may be isolated from fluid cultures.

The susceptibility of various animals for the toxin is briefly as follows: Chickens and dogs are non-susceptible to subcutaneous injection of 100,000 guinea pig M.L.D.'s; 5 guinea pig M.L.D. subcutaneously are fatal to a rabbit in 72 hours; 1,000 M.L.D. for a rat in 7 days; 100,000 M.L.D. for a pigeon in 24 hours. Contrary to the observation of Theiler, mice are susceptible; a toxin which was fatal for guinea pigs in a dilution of 1:20,000 fatally poisoned mice in a dilution of 1:50,000. The ratio of the M.L.D. by subcutaneous injection to the M.L.D. *per os* is 1:1,000 for guinea pigs, 1:200 for mice and >1:3,000 for rabbits. Suitable suspensions of the *Cl. botulinum* Type D are not agglutinated by any of the type sera available in this laboratory but are readily clumped by a homologous antiserum.

The anaerobe isolated from lamziekte carrion is a non-ovolytic and non-sarcolytic organism which produces a highly potent neurotoxin. Pharmacologically the poison acts on small laboratory animals like the botulinum toxin. It is not neutralized by any of the known Type A, B and C antitoxins. The bacterium should therefore be designated *Cl. botulinum* Type D (Theiler and Robinson<sup>1</sup>) and should be definitely separated from the ovolytic *Cl. paratubulinum* Type A and B.

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### Susceptibility of Macacus Rhesus Monkeys to Botulinum Toxin Type B, C and D.

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Thus far no cases of botulism due to *Cl. botulinum* Type C have been reported and nothing definite concerning the susceptibility of man to this toxin is known. On the other hand since the great sus-