before each determination, and that each one felt that no disturbing factors influenced the test, as complete physical and mental repose had been attained.

The results in 2 of the subjects (M.H.S. and J.B.L.) are similar. The first dose produced a definite rise which was maintained after the second dose. Determinations were not made after the third and fourth doses, but the basal rate had dropped to the pre-experimental level after the fifth and last dose. In one (J.B.L.), this basal level was maintained in the post-experimental control determination; in the other (M.H.S.), this determination had dropped sufficiently below the supposed basal level to be significantly beyond the limit of experimental error. The rates of the third subject (T.B.L.) show no significant change except a tendency to drop, though hardly beyond the limit of possible error; in the control determination, however, there was a marked drop, as had occurred in M.H.S. The significance of this drop is not apparent.

There was no significant change in the basal temperature, pulse-rate or blood-pressure of any of the subjects. Although the general effects varied somewhat in the different individuals, they were, on the whole, the same as has previously been reported.[1, 2, 3]

It was not expected that the basal level would be as low as was found. Whether this influenced the rise in rate and whether it accounted for the failure of this rise to be maintained, is not known. It seems reasonable to expect individuals with a normal level (from 10.0% minus to 10.0% plus) to show the same degree of rise; whether the rate would drop to the previous level under continued medication or maintain its level as long as the drug was ingested, is difficult to prophesy. This is the next problem planned for investigation.

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Clostridium Botulinum Type E.

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Two cultures of *Clostridium botulinum* were sent to this laboratory in March, 1936, by Dr. L. Bier of the Bacteriologic Institute at Dniepropetrowsk, Ukraina, U.S.S.R. Toxin-neutralization tests had suggested a new type. The original cultures were toxic for

mice in doses of 0.001 cc. and antitoxin of types A, B, C and D failed to protect against the the toxin.

The organism is a Gram positive, motile, granular, pleomorphic rod and forms oval, subterminal spores which swell the rods very slightly. In liver-agar shake-cultures small, disc-like colonies with or without small polar fluffs are formed. Colonies on the surface of glucose blood plates are non-hemolytic, greyish, translucent, smooth and flat with a marked tendency toward confluence.

These cultures are not ovolytic and do not liquefy coagulated egg-white even after a month's growth. In beef heart medium there is slight reddening of the meat and a large volume of gas is evolved. Brain is not blackened or digested. In milk, slight acid is produced but the casein is not attacked. Slight liquefaction of gelatin occurred after 23 days. The peptolytic properties are extremely low and the Sörenson figure was 2.00 after 21 days' growth.

Dextrose, levulose, maltose, sucrose, arabinose, xylose and adonite are fermented with little or no gas production in a medium composed of 0.3% Liebig's extract, 0.5% Difco peptone, and 1% carbohydrate. Lactose, rhamnose, galactose, dextrin, raffinose, glycerin, salicin, mannite, inulin and dulcite are not fermented.

The thermal resistance of the spores is extremely low. Spores were destroyed in a suspension in buffer solution (pH 7.4) containing 5 million spores per cc. after heating at 100°C. for 2 minutes or 80°C. for 6 minutes. A suspension containing 50 million spores per cc. failed to show growth after 5 minutes at 100°C. or after 40 minutes at 80°C.

Reciprocal agglutinin-absorption tests showed these 2 cultures to be identical. There was no agglutination with either H or O antisera representative of various groups of *Cl. parabotulinum* and *Cl. botulinum*.

Toxin-production is variable and the cultures tend to become nontoxigenic. Formation of toxin was more constant at room temperature than at 37°C., although growth was equally rapid and luxuriant at either temperature. The most potent toxin obtained was produced in a medium composed of 1% tryptone (Difco) and 2% glucose at pH 7.4.

The MLD of toxin for 350 gm. guinea pigs on subcutaneous injection was 0.02 cc. A 2100 gm. rabbit was killed within 18 hours by subcutaneous injection of 10 guinea pig MLD and 0.025 MLD was fatal to 18 gm. mice within 96 hours. Chickens were not susceptible to injection of 2000 guinea pig MLD. By feeding, approximately 150 times as much toxin was required to kill guinea pigs as by injection and about 200 times as much for mice. Feeding of

1000 guinea pig MLD to 2000 gm. rabbits was without effect. A 3000 gm. monkey showed no symptoms when fed approximately 500 MLD of toxin. Ten days later the same monkey received 2500 MLD and died within 16 hours. A 2500 gm. monkey died within 21 hours when about 2000 MLD were given by mouth. The susceptibility of monkeys to this toxin is somewhat lower than to toxin of *Cl. botulinum* type B which killed in a dose of 100 MLD.

Toxin-antitoxin tests made by injection of mice and guinea pigs showed that antitoxin of types A, B, C and D in doses adequate to protect against 250 to 170,000 MLD of homologous toxin failed to neutralize 2 to 5 MLD of this toxin. Antitoxin for these cultures was produced by injection of rabbits with toxoid and 0.05 cc. protected against at least 10 lethal doses for mice of the homologous toxin. This antitoxin in 0.5 cc. amounts failed to protect against 2 to 3 fatal doses of toxin of types A, B, C and D. Hence, these strains must represent a new type.

The organism closely resembles *Cl. botulinum* types B, C and D in morphology, in failure to attack protein, and in other cultural reactions. The potent neurotoxin acts on small laboratory animals in the same manner as do toxins of other types. It resembles toxins of *Cl. botulinum* types B, C and D in that chickens possess a high degree of immunity to it. Like toxin of *Cl. botulinum* type B it is fatal to monkeys by mouth and thus may play a rôle in botulism in humans. It is not neutralized by antitoxins of types A, B, C or D. The designation *Clostridium botulinum* type E is, therefore, proposed for this organism. Topley and Wilson, Second Edition, 1936, p. 688, have designated the *Cl. parabotulinum equi* of Theiler and Robinson as *Cl. botulinum* type E. Robinson[1] has conclusively shown that the organism of equine botulism belongs to type C and his results were confirmed by Graham and by the authors.

[1] Robinson, E. M., Sixteenth Rep. Director Vet. Serv. and Animal Industry, Union S. Africa, 1930, p. 107.