The following table gives a comparison of the various C types and the D one as regards their cultural characteristics etc. It will be seen that any differentiation on them alone would be very difficult though there are some features in which they differ from the A and B types of the Cl. botulinum which are given for the sake of comparison.

## MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF THE BOTULINUS TYPES.

Adapted from Table 1 in "Der botulismus der Haustiere" Theiler and Robinson (1927).

| Type | Motility | Morphology | Staining | Chopped meat medium | Hibler medium | Glucose agar shake | Liver ager shake | Gelative | .1% agar | Serum broth | Glucose borth | Litmus milk | Sugar media |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cl.botulinum A (Type A 223 Lister Inst. Cl.botulinum B (Type B95, Lister Inst.) | Motile under anarobic conditions | Length 4 to 9μ Breadth .9 to 1.2μ Oval spores usually terminal | Gram + easily decolorised | Gas rancid Small turbidity Meat blackened. | As in chopped meat | Gas, lens shaped colonies formed | Gas. Lens shaped colonies formed | Liquefied | Uniform turbidity. | Turbid | Gas, turbidity | Not coagulated. Reduced | Ferment glucose, lactose, levulose, maltose, dextrin, glycerol and salicin |
| Cl.botulinum C (From R.Graham Univ. of Illinois U.S.A.) | -do- | Length 5 to 8μ Breadth .9μ Spores oval and terminal. Filaments formed | -do- Gram - forms soon appear | Gas. No turbidity. Meat not blackened | Gas turbidity. No blackening. | No gas. Fluffy colonies like bits of cotton wool | Gas, fine fluffy colonies like cotton wool | Not liquified | -do- | Turbid Growth settles out soon | No gas. Flocculent growth which settles out | Acid coagulated. | As A and B but dextrin not fermented |
| B.parabotulinus (Seddon) American Museum of Natural History) | -do- | L. 3 to 8μ Br. 1μ. Spores oval and subterminal | -do- Mainly Gram - in old cultures | -do- | -do- | -do- | -do- | -do- | -do- | -do- | -do- | -do- | No sugars fermented |
| Cl.botulinum D (Theiler and Robinson). Formerly Cl.parabotulinum bovis | -do- | L. 4 to 8μ Br. .8 to 1μ Spores oval and terminal short chains frequent | -do- | -do- Sweetish smell | -do- | -od- | -do- | -do- | -do- | -do- | -do- | -do- | No gas. Ferments glucose levulose, sucrose, lactose, maltose, glycerin, inosite and galactose |
| Cl.botulinum C (equine type) Formerly Cl.parabotulinum equi | -do- | L. 4 to 8μ Br. .8 to 1μ Spores terminal. Chains frequent | -do- | -do- | -do- | -do- | -do- | -do- | -do- | -do- | -do- | -do- | Gas and acid in glucose, levulose, lactose, maltose glycerin. |
| Cl.botulinum 334 (A type probably identical with the C one). | | -do- | -do- | -do- | -od- | -do- | -do- | -do- | -do- | -do- | -do- | -do- | |

COMPARATIVE TOXICITY OF THE BOTULINUS TYPES WITH APPROXIMATE MINIMUM LETHAL DOSES.

Adapted from Table III in "Der botulismus der Haustiere (1927) with additions.

| Type | Man | Ape | Horse | Ox | Sheep | Goat | Pig | Dog | Cat | Rabbit | Guinea pig | White rat | House rat | House mouse | Fowl | Duck | Pigeon and Ostrich |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cl.botulinum A (Writer's type was A.223 Lister Institute. | Very toxic | .01 to .001 c.c. subc. | .2 c.c. lethal in 4 days | about 5 c.c. subcut. | .01 c.c. subcut. | .01 c.c. subc. | Not susceptible | Not susceptible | .1 to 1 c.c subc. (von Ermengem. | .0001 to .001 c.c. cubc. 1-3 c.c. per os | .0001 to .001 c.c subcut. .1 to 1 c.c. per os | Very slightly susceptible. | As white rat | .0001 c.c. subc. | Large doses per os | Large doses per os. | .1 c.c. to .5 c.c. subc. in pigeon |
| Cl.botulinum B (B 95 Lister Institute) | -do- | -do- | .05 c.c lethal in 12 days 1 | -do- | -do- | -do- | -do- | -do- | -do- | -do- | -do- | - | - | -do- | -do- | - | - |
| Cl.botulinum C (From R. Graham University of Illinois, U.S.A.) | - | Small doses subc. large doses per os | 120 c.c culture per os | 2.5 c.c subc. About 200 gms. culture per os not fatal. | - | - | -do- | -do- | Not susceptible | -do- | .001 c.c subcut. 1 c.c. per os | .001 c.c. subc. | - | .001 c.c. subc. | 20 c.c per os in some cases | - | - |
| B.parabotulinus (Seddon) American Museum of Natural History. | - | - | .04 c.c subc. | 2.5 c.c subc.12 c.c.per os gave no result | .02 c.c subc. 2.5 c.c per os | about .01 c.c. subc. | -do- | -do- | -do- | .001 c.c. subc. | .001 c.c subcut. .1 to 1 c.c. per os. | - | - | .0001 to .001 c.c. subc. | 30 c.c or more per os | - - | - |
| Cl.botulinum D (Theiler and Robinson) (Cl.parabotulinum bovis). | - | As in Cl.bot. C. | .005 c.c per kilo subc. | 5 c.c. culture per os | .0001 c.c. per Kilo subc. | .0001 c.c per Kilo subc. | -do- | -do- | -do- | .001 c.c per kilo subc. 1 to 3 c.c per os | .001 c.c per kilo .1 to 1 c.c. per os | 1 c.c subc. | - | .0001 c.c. subc. | No result | 40 c.c culture per os | Pigeon 2 c.c. subcut. Ostrich 20 c.c. per os. |
| Cl.botulinum C (equine type) (Cl.parabotulinum equi). | - | - | .015 c.c subc.100 c.c filt. per os 10 c.c.culture per os | .05 c.c subc. | .01 c.c subc. | .01 c.c. subc. | -do- | -do- | -do- | -do- | -do- | 1 c.c. subc. No result per os | 1c.c. subc. No result | -do- | 5 c.c subc. no result. | No result | - |
| Cl.botulinum 334 (A type probably identical with th C one | - | - | - | - | -do- | -do- | - | - | - | .001 c.c. subc. | -do- | - | - | -do- | - | - | - |

(2) Comparison by toxicity for different animal species.

While realizing that toxin antitoxin and serological tests form the only comparative standard of any real value, it is of interest to note the variation in toxicity of the different varieties of the Cl. botulinum C and D types. One has to assume that the various authors are referring to recently isolated cultures, as in any type the toxicity is liable to fall off markedly in the course of time, even when constantly subcultured.

In the original article of Bergtson (1922) only the effects of the toxin of Cl. botulinum C on certain animal species were mentioned. These included the rabbit, guinea pig, rat, mouse, monkey, pigeon and chicken. In the article of Graham and Boughton (1923) however, also in relation to the C type, the effects on a bigger variety of animal species were described. As compared with the results of Bergtson, the latter authors found that larger doses were required to produce symptoms in fowls, in one case .71 c.c. of meat wash culture per os only apparently killing because the chicken's resistance had been reduced by roup. A feature of the C type of Graham and Boughton as described, was the large amount of culture necessary to produce symptoms in cattle and horses, and from the experimental evidence these animals must be considered as comparatively speaking resistant to that particular toxin.

In his article on the Bacillus parabotulinus, Seddon (1922) gives the toxicity of cultures of the organism for a number of animal species but leaves out some of importance such as the pig, goat, dog, cat, rat and mouse. Theiler and Robinson (1927) give the toxicity of the Cl. parabotulinum bovis or Cl. botulinum D as it should preferably be called, for the domesticated animal species and small laboratory animals, leaving out the monkey however. This want has been recently supplied by Meyer and Gunnison (1928), so that the

list is fairly complete. The marked toxicity for cattle in comparison with horses is noteworthy.

In their description of Cl. parabotulinum equi, Theiler and Robinson (1928) give the toxicity for all available species of domesticated and laboratory animals. This organism, as has been mentioned elsewhere (see comparison by toxin antitoxin tests), is not distinguishable from the Cl. botulinum C of Bergtson and Graham and Boughton. The extreme toxicity for horses as compared with other animals, may however, eventually cause it to be classified as a variety of the C type. It has not yet been tested on monkeys so no comparison can be made with other C types from that point of view.

Recently, as mentioned above, Meyer and Gunnison (1928) have tried both the C and D types as to their toxicity for macacus monkeys, and express the opinion that their toxicity is much lower than that of either the A or B type of the Cl. botulinum and infer from that that their toxicity for man is probably low. So far no outbreaks of botulism in man have been traced to toxins of the C ar D types. It is probable that in South Africa such outbreaks would be heard of frequently if these types were very toxic for man, as meat is frequently eaten half cooked or almost raw and from animals which had been dead for some hours before being skinned.

(3) <u>Comparison by means of agglutination tests</u>.

The serological classification of the A and B types of the Cl. botulinum has been carefully studied by Schoenholz and Meyer (1925), who employed the precipitin, complement fixation and agglutination tests, the latter test being used on a large scale. The results were very satisfactory and proved of great value in identifying types.

Pfenninger (1924) used the agglutination test in the classification of the Cl. botulinum C types and the B para-

botulinus of Seddon. He compared them with the A and B types, the results bearing out in a striking manner those obtained by the toxin antitoxin tests.

The writer experienced much difficulty at first in the immunizing of rabbits against the washed cultures of the various botulinus types. It was not found for instance, that washing three times in normal saline and subsequently heating to 60°C for one hour was always effective in freeing the bacteria from toxin. With strains of low toxicity, not much difficulty was experienced, but when it came to those with powerful toxins, much difficulty was experienced at first. The writer had many setbacks in his earlier attempts at immunization, owing to rabbits dying of botulism after the second or third inoculation with bacteria from highly toxic strains. Later, formalized cultures were used (.4% Formalin) and even without washing, these proved very useful for immunization. The formalin may cause clumps to form, particularly in unwashed cultures, but they do not interfere with the immunizing properties.

In order to obtain the bacteria in bulk for inoculation of animals and for serological tests, the writer used the shell broth method described by McEwen (1926). In this method, which is a modification of the methods using particles of tissue in a fluid medium, the meat is put in a parchment diffusion shell, so that it is not actually mixed with the broth or other fluid medium. The surface of the broth which is usually put up in flasks, is covered with a layer of liquid paraffin. The presence of the meat in the diffusion shell appears to produce the requisite anaerobic conditions, and one gets a heavy growth without the drawback of having meat particles mixed with the bacteria.

The writer has used goats in his later immunization work, on account of the smaller risk of losing them, more especially from intercurrent infections, and has found them to give good antibacterial sera. They have the additional

advantage of giving larger quantities of serum.

The goats were given five subcutaneous inoculations at weekly intervals of a dense emulsion of bacteria, commencing with 1 c.c. and working up to 5 c.c. The sera remained effective for at least six months when kept in cold storage.

For the tests themselves the writer has not made much use of the ordinary macroscopic agglutination method as generally carried out. The reason for this is that emulsions of the botulinus types, more particularly the C ones, show a marked tendency to form small bacterial masses which settle out by themselves fairly quickly, leaving the medium clear. This tendency was less marked with the A and B types and naturally interfered somewhat with tests done by the ordinary macroscopic method.

It was, therefore, decided to adopt the rapid agglutination method of Huddleson (1926) and referred to by Noble (1927). In the writer's hands this method has given excellent results. The technique is simple, and the actual tests are carried out on a plate of glass divided into small squares instead of using tubes. In the first tests carried out by Huddleson using this method, tubes were used but were discarded in favour of a flat glass surface. Using a sheet of glass, one can see the clumps form more readily and the results can be read much sooner.

The amounts of serum used are the same as for the usual macroscopic test, but a much smaller quantity of a dense emulsion of the bacteria is used. The emulsion should be so dense that using the Gates nephelometer (1921) the platinum loop disappears from sight at a depth of 4 m.m. The bacteria are suspended in a 12% solution of sodium chloride. After mixing the serum and emulsion, clumping often takes place at once, even without warming, but one should warm the plate of glass up to blood heat over a flame and

and then read the result. In less than five minutes the agglutination is usually complete but the test should be read again after fifteen minutes. A series of comparative tests will now be given in tables:

Table 1.

Cl. botulinum D antiserum (Rabbit).

| Emulsion | 1/25 | 1/100 | 1/200 | 1/500 | 1/1000 | 1/2000 | 1/5000 | 1/10000 | 1/20000 |
|---|---|---|---|---|---|---|---|---|---|
| Cl.bot. (333) | + | ± | - | - | - | - | - | - | - |
| Cl.parabot bovis | ++ | ++ | ++ | ++ | ++ | ++ | ± | - | - |
| " bot (D) | | | | | | | | | |
| " bot C | + | ± | - | - | - | - | - | - | - |
| B.parabot (Seddon) | + | ± | - | - | - | - | - | - | - |
| Cl.parabot equi (C) | +± | + | + | ± | - | - | - | - | - |

From this table it will be seen that Cl. botulinum D antiserum will not agglutinate any except the homologous bacterial emulsion to any marked degree. The organism referred to in the table as Cl. botulinum 333 was obtained from the carcase of an animal which had died of lamsiekte at Armoedsvlakte, Bechuanaland (see toxin list). From cross toxin antitoxin tests it appeared to be different to Cl. parabotulinum bovis D and the agglutination test shows that they are apparently different organisms.

In the following tables the following symbols have been used:

++ = very marked agglutination

\+ = definite agglutination.

± = partial "

\- = no "

Table 2.

Cl. botulinum D emulsion.

| Antisera | 1/50 | 1/100 | 1/200 | 1/500 | 1/2000 | 1/4000 | 1/8000 | 1/16000 | 1/32000 |
|---|---|---|---|---|---|---|---|---|---|
| Cl.parabot bovis (Cl bot D or | ++ | ++ | ++ | ++ | ++ | ++ | + | ± | - |
| B.parabotulinus (Seddon) | ++ | ++ | + | ± | - | - | - | - | - |
| Cl. botulinum C | ++ | ++ | ± | - | - | - | - | - | - |
| Cl. botulinum B | ++ | + | ± | - | - | - | - | - | - |

In this table an emulsion of the Cl.parabot. bovis (Cl. botulinum D) was tested against four different antisera. The homologous antiserum was the only one to clump the bacteria to any marked extent.

Table 3.

Cl. botulinum C emulsion.

| Antisera | 1/50 | 1/100 | 1/200 | 1/400 | 1/800 | 1/1600 | 1/3200 | 1/6400 | 1/12800 |
|---|---|---|---|---|---|---|---|---|---|
| Cl.botulinum C | ++ | ++ | ++ | ++ | ++ | ++ | +- | ± | ± |
| B.parabotulinus (Seddon) | ++ | ++ | + | ± | - | - | - | - | - |
| Cl.parabot. bovis (D) | ++ | ++ | ++ | + | ± | - | - | - | - |
| Cl.botulinum B | ++ | ++ | + | ± | - | - | - | - | - |

The antisera used for these tests were the same as those used in table 2 and were made by immunising goats. Again the homologous serum was the only one to agglutinate to a marked degree. Evidence of some degree of group agglutination is seen in these tests.

Table 4.

Cl. botulinum B emulsion.

| Antisera | 1/50 | 1/100 | 1/200 | 1/400 | 1/800 | 1/1600 | 1/3200 | 1/6400 | 1/1280 |
|---|---|---|---|---|---|---|---|---|---|
| Cl.botulinum B | ++ | ++ | ++ | ++ | ++ | ++ | + | ± | - |
| " " C | ++ | + | ± | -- | - | - | - | - | - |
| " " D (bovis) | + | + | ± | - | - | - | - | - | - |
| B.parabotulinus (Seddon) | + | ± | - | - | - | - | - | - | - |

In this table the evidence of group agglutination is less marked than in the two previous tables. Some variation is to be expected when using different emulsions and emulsions of the same organism prepared at different times may give slightly varying results.

Table 5.

Cl. botulinum B emulsion.

| Antisera (goat) | 1/50 | 1/100 | 1/200 | 1/400 | 1/800 | 1/1600 | 1/3200 | 1/6400 |
|---|---|---|---|---|---|---|---|---|
| Cl. parabot bovis or " botulinum D | ++ | + | ± | - | - | - | - | - |
| B.parabotulinus (Seddon) | ++ | + | ± | - | - | - | - | - |
| Cl. botulinum C | + | ± | + | - | - | - | - | - |
| Cl. parabot. equi (C) | ± | - | - | - | - | - | - | - |
| Cl. bot A | + | + | ± | - | - | - | - | - |
| Cl. " B | ++ | ++ | ++ | ++ | ++ | + | ± | - |

The emulsion in this case was not apparently as agglutinable as that in table 4 but the results correspond very closely.

Table 6.

B. parabotulinus (Seddon) emulsion.

| Antisera (Rabbit) | 1/50 | 1/100 | 1/200 | 1/400 | 1/800 | 1/1600 | 1/3200 | 1/6400 | 1/12800 | 1/25600 |
|---|---|---|---|---|---|---|---|---|---|---|
| Cl. parabot. (bovis) D | ++ | ++ | ++ | ++ | + | ± | - | - | - | - |
| Cl. parabot. equi (C) | ++ | ++ | ++ | ++ | +, | ± | - | - | - | - |
| Cl. botulinum C (1) | ++ | ++ | + | ± | - | - | - | - | - | - |
| Cl. botulinum C (2) | ++ | ++ | ++ | ++ | + | ± | - | - | - | - |
| B. parabotulinus (Seddon) | ++ | ++ | ++ | ++ | ++ | ++ | ++ | + | ± | - |

Evidence of group agglutination is well marked in this table. The Cl. botulinum C sera were from two different rabbits and one gave a much stronger agglutination than the other.

Table 7.

B. parabotulinus (Seddon) emulsion.

| Antiserum (Goat) | 1/50 | 1/100 | 1/200 | 1/500 | 1/1000 | 1/2000 | 1/4000 | 1/8000 | 1/16000 | 1/32000 |
|---|---|---|---|---|---|---|---|---|---|---|
| B.parabotulinus (Seddon) | ++ | ++ | ++ | ++ | ++ | ++ | ++ | + | ± | - |
| Cl.botulinum C | ++ | ++ | + | ± | - | - | - | - | - | - |
| Cl.botulinum bovis D (bovis) | ++ | + | + | ± | -- | - | - | - | - | - |
| Cl.botulinum equi C | ++ | ++ | ++ | ++ | + | ± | - | - | - | - |

The results are more or less the same as in table 6, except that the equi antiserum gave a stronger reaction than the C

In table 8 a Cl. botulinum C emulsion was used, this time including an equi antiserum.

Table 8.

Cl. botulinum C emulsion

| Antiserum (Goat) | 1/50 | 1/100 | 1/200 | 1/400 | 1/800 | 1/1600 | 1/3200 | 1/6400 |
|---|---|---|---|---|---|---|---|---|
| Cl.botulinum C | ++ | ++ | ++ | ++ | ++ | + | ∓ | - |
| B. parabotulinus (Seddon) | + | + | ∓ | - | - | - | - | - |
| Cl. botulinus D (bovis) | ++ | ++ | + | ∓ | - | - | - | - |
| Cl. botulinus C (equi) | ++- | ++ | + | ∓ | - | - | - | - |

The equi anti-serum did not agglutinate the emulsion as strongly as the C

Table 9.

Cl. botulinum C (equi) emulsion.

| Antiserum (goat) | 1/50 | 1/100 | 1/200 | 1/400 | 1/800 | 1/1600 | 1/3200 | 1/6400 |
|---|---|---|---|---|---|---|---|---|
| Cl. botulinum D (bovis) | ++ | ++ | ++ | ∓ | - | - | - | - |
| B.parabotulinus (Seddon) | ++ | ++ | ++ | + | ∓ | - | - | - |
| Cl.botulinum C (equi) | ++ | ++' | ++ | ++ | ++ | ++ | + | ∓ |
| Cl. botulinum C | ++ | ++ | ++ | ++ | + | ∓ | - | - |

The results in this table show the close relationship of the C and C (equi) types.

Table 10.

Cl. botulinum 334 (C type)

| Antiserum (Goat) | 1/50 | 1/100 | 1/200 | 1/400 | 1/800 | 1/1600 | 1/3200 | 1/6400 |
|---|---|---|---|---|---|---|---|---|
| Cl. botulinum D (bovis) | ++ | ++ | + | ∓ | - | - | - | - |
| Cl. botulinum C (equi) | ++ | ++ | ++ | ++ | ++ | + | ∓ | - |
| Cl. botulinum C | ++ | ++ | ++ | ++ | ++ | + | ∓ | - |
| B. parabotulinus (Seddon) | ++ | ++ | ++ | + | ∓ | - | - | - |

This table shows the close relationship of the C, C (equi) and 334 types.

As shown by toxin antitoxin tests, Cl. botulinum 334 is definitely a C type of the Cl. botulinum, and the tests in Table 10 therefore confirm the relationship.

Table 11.

Cl. botulinum B antiserum (goat).

| Emulsion | 1/50 | 1/100 | 1/200 | 1/400 | 1/800 | 1/1600 | 1/3200 | 1/6400 |
|---|---|---|---|---|---|---|---|---|
| Cl. botulinum B | ++ | ++ | ++ | ++ | ++ | ++ | + | ± |
| " " A | ++ | +' | ± | - | - | - | - | - |
| " " C | ++ | ++ | + | ± | - | - | - | - |
| " " C (equi) | ++ | + | ± | - | - | - | - | - |
| " " D (bovis) | ++ | + | ± | - | - | - | - | - |
| B. parabotulinus (Seddon) | ++ | + | ± | - | - | - | - | - |

With Cl. botulinum A antiserum (goat) only the A emulsion was strongly agglutinated.

Table 12.

Cl. botulinum C antiserum (goat)

| Emulsion | 1/50 | 1/100 | 1/200 | 1/400 | 1/800 | 1/1600 | 1/3200 | 1/6400 | 1/12800 |
|---|---|---|---|---|---|---|---|---|---|
| Cl. botulinum A | + | ± | - | - | - | - | - | - | - |
| " " B | ++ | + | ± | - | - | - | - | - | - |
| " " C | ++ | ++ | ++ | ++ | ++ | ++ | ++ | + | ± |
| " " C (equi) | ++ | ++ | ++ | ++ | ++ | ++ | +' | ± | - |
| " " D (bovis) | ++ | +, | ± | - | - | - | - | - | - |
| B. parabotulinus (Seddon) | ++ | + | ± | - | - | - | - | - | - |

Table 13.

Cl. botulinum C (equi) antiserum (goat)

| Emulsion | 1/50 | 1/100 | 1/200 | 1/400 | 1/800 | 1/1600 | 1/3200 | 1/6400 | 1/12800 |
|---|---|---|---|---|---|---|---|---|---|
| Cl. botulimum A | ++ | + | ± | - | - | - | - | - | - |
| " " B | ++ | ++ | + | ± | - | - | - | - | - |
| " " C | ++ | ++ | ++ | ++ | ++ | + | ± | - | - |
| " " C (equi) | ++ | ++ | ++ | ++ | ++ | ++ | + | ± | - |
| " " D (bovis) | ++ | ++ | +' | ± | - | - | - | - | - |
| B. parabotulinus (Seddon) | ++ | ++ | ++ | ++ | + | ± | - | - | - |

## Table 14.

Cl. botulinum D (bovis) antiserum (goat)

| Emulsion | 1/50 | 1/100 | 1/200 | 1/400 | 1/800 | 1/1600 | 1/3200 | 1/6400 | 1/12800 |
|---|---|---|---|---|---|---|---|---|---|
| Cl.botulinum A | + | ± | - | - | - | - | - | - | - |
| " " B | ++ | + | ± | - | - | - | - | - | - |
| " " C | ++ | + | ± | - | - | - | - | - | - |
| " " C (equi) | ++ | ++ | ++ | + | ± | - | - | - | - |
| " " D (bovis) | ++ | ++ | ++ | ++ | ++ | ++ | ++ | + | + |
| B. parabotulinus (Seddon) | ++ | ++ | ++ | + | ± | - | - | - | - |

## Table 15.

B. parabotulinus (Seddon) antiserum (goat).

| Emulsion | 1/50 | 1/100 | 1/200 | 1/400 | 1/800 | 1/1600 | 1/3200 | 1/6400 |
|---|---|---|---|---|---|---|---|---|
| Cl. botulinum A | ++ | + | ± | - | - | - | - | - |
| " " B | + | ± | - | - | - | - | - | - |
| " " C | ++ | ++ | + | ± | - | - | - | - |
| " " C (equi) | ++ | ++ | ++ | + | - | - | - | - |
| " " D (bovis) | ++ | ++ | +' | ± | - | - | - | - |
| B.parabotulinus (Seddon) | ++ | ++ | ++ | ++ | ++ | ++ | ++ | + |

## CONCLUSIONS.

(1) From the comparative agglutination tests carried out, it will be seen that there is a definite group relationship between certain of the various types dealt with. The close relationship of the Cl. botulinum C (American strain) and the Cl. botulinum C (equi) is shown as well as that of the Cl. botulinum (334) to the other two as demonstrated by toxin antitoxin tests.

(2) The identity of the B. parabotulinus of Seddon with any of the other types was not demonstrated and it would appear that is a variant of the C type.

(3) The value of the shell broth medium for obtaining large amounts of bacterial emulsions of the C and D types in particular is emphasized.

(4) The rapid agglutination test of Huddleson (1926) can be used for the bacteria of Cl. botulinum type and has definite advantages over the longer methods.