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Gas-Gangrene-Toxin Production.

G. B. REED, J. H. ORR AND MARY C. BAKER. (Introduced by R. G. Sinclair.)

From Queen's University, Kingston, Canada.

Since Bull's original demonstration that *Cl. welchii* is toxigenic it has been customary to cultivate this and other gas-gangrene anaerobes for toxin-production in chopped-meat media, rich infusion-broths or serum-broths. It seems evident from the literature that many workers have found these methods to give highly variable yields of toxin. In our experience different batches of such media made in an apparently similar manner, inoculated with the same strain of organism and handled in as nearly as possible an identical manner, give very different yields of toxin. The recent experience with the production of diphtheria toxin has indicated that more consistent yields may be obtained by substituting for complex infusions, media of known, or for the most part known, chemical composition.

After several preliminary trials the following medium was found to give fairly high yields of toxin which are consistent from batch to batch with all the toxin-producing members of the gas-gangrene group of organisms:

NaCl	2.	g
MgSO ₄ 7H ₂ O	0.02	"
Na ₂ HPO ₄ 12H ₂ O	5.76	"
KH ₂ PO ₄	0.24	"
Gelatin, Difco	50.	"
Peptone, Bacto	10.	"
Glucose	2.	"
Water	1000	cc

pH 7.7, Autoclaved at 15 lb.

The most consistent results have been obtained with freshly prepared and autoclaved media. While small inocula give good growths, more uniform toxin-yields have followed the use of inocula consisting of 3% of a 5 to 6 hour culture in the same medium.

Many modifications of this medium have been tried, particularly various proportions of gelatin and peptone. Variations of 1 to 10% of gelatin and 0.1 to 10% of peptone give fair yields of toxin but with a maximum at about 5% gelatin and 1% of peptone. Bacto peptone is slightly superior to several others tested. Fair yields of toxin are obtained with 5% of gelatin and no peptone. Larger concentrations of glucose and other sugars, fermentable by the organism in question, give somewhat more rapid growth but the yield of toxin

is conspicuously lower probably due to the greater acid-production. Variation in the phosphate-content has relatively little influence provided the buffer value is sufficient to prevent a fall in pH from the initial 7.7 to not less than 6.2 to 6.0 in 18 to 24 hours.

Relative viscosity and formol titration indicate that a rapid hydrolysis of the gelatin begins 1 to 2 hours after inoculation and continues at a rapid rate beyond the time toxin production is at a maximum, 18 to 24 hours. *Cl. welchii* cultures, in this medium, transform 16 to 18% of the gelatin-nitrogen to amino-nitrogen by the time maximal toxin-production is reached, whereas cultures of *Cl. septicum* at the time of maximal toxin-production show slightly less than half this amount of amino-nitrogen.

Measurable amounts of toxin are produced after 6 to 8 hours' incubation, the concentration increases conspicuously up to about 18 hours, shows relatively little change for the next 4 to 6 hours, then gradually decreases until it practically disappears after 2 to 3 days' incubation of the culture. The toxin may be filtered through Seitz or sintered glass (Jena G5 on 3) filters with comparatively slight loss of toxicity, compared with centrifuged cultures, provided the first 20 to 30 cc of filtrate, in the case of small filters, is discarded.

Toxins from this medium may be satisfactorily precipitated with ammonium sulphate and dried. Good toxoids are made by a relatively short period of formolization.

Various toxigenic strains of the 3 principal species of gas-gangrene organisms grown in this medium produce the following concentrations of toxin, estimated as hæmotoxin. Hæmotoxin-titration end-point was taken as the dilution of toxin that will hæmolyze 50% of a 2% suspension of rabbit's red cells:

<i>Cl. welchii</i>	1-1000 to 1-4000
<i>Cl. septicum</i>	1-100 to 1-200
<i>Cl. novyi</i>	1-100 to 1-200