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DIPHTHERIA TOXIN PRODUCTION ON MEDIUM PREPARED BY HARTLEY'S MODIFICATION OF DOUGLAS'S METHOD.

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Using Hartley's modification of the Douglas medium (Hartley, 1922 [1]) two closely allied methods for the large scale production of this medium have been developed. The principle of these methods consists in the digestion of horse muscle by an extract of trypsin prepared by the method of Cole and Onslow (1914 [2]). For large quantities of medium the digestion is carried out in a hundred litre cauldron provided with a steam jacket, the initial temperature of 45° C. being allowed to drop to 40°, and then maintained at this level for the remainder of the digestion. Under these conditions four hours' digestion gives a valuable medium for the preparation of diphtheria toxin. For small quantities of media the digestion may be carried out conveniently in earthenware vessels in the incubator, an initial temperature of 45° being allowed to drop to 37°, and maintained at this level for the remainder of the digestion. Under these conditions a six hours' digestion gives a medium of similar composition to the above, and of equal value for the production of diphtheria toxin. Close attention to the details of the digestion is necessary, if the ultimate value of the medium is to be the maximum for the production of toxin. Over one thousand litres of high-grade toxin have been made from media prepared by these methods. For the preparation of this toxin Park Williams No. 8 strain was employed. The parent strain was sub-cultured on to four 100 c.c. quantities of medium, and these sub-strains maintained by sub-inoculation on similar quantities of medium throughout the work. With every large quantity of medium prepared, a small number of "starter" bottles containing 100 c.c. medium was made and the sub-strains sown on four of these. Seventy-two hour culture filtrates of these strains were examined for toxin after resowing on fresh medium. The sub-strain giving the highest value toxin for the particular large quantity of medium was used for inoculating the remainder of the brew distributed in double Winchester quart bottles, one litre per bottle.

During part of 1922 somewhat over 1000 litres of toxin were prepared; the lowest Lr/500 value was 0·0004, and the highest 0·00015, the average being about 0·00025.

The toxicity of the various filtrates was tested for the Lr/500 value by the intracutaneous method of Romer and Sames (1909 [3]). Although no definite relationship exists between the Lr/500 value and the M.L.D. value, it has been found for medium prepared by the above methods, and employing the strain used in the present work, that toxins which had an Lr/500 value of ·0002 generally killed a guinea-pig weighing 250 grams in doses of one five-hundredth of a c.c. Toxins with an Lr/500 value of ·00025 and ·0003 had an M.L.D. of nearly the same value. No volumes of useless toxin, frequently met with when using the older types of "peptone" medium, were encountered. During this work evidence has accumulated which suggests that there are critical periods during the digestion of horse muscle which must be avoided if the ultimate value of the medium is to be the maximum for the production

of toxin. It is suggested that similar unsatisfactory periods may occur during the digestion of other proteins used in the production of commercial "peptones," and that this possibility may contribute to the variable results obtained when these peptones are employed for the production of diphtheria toxin.

Toxin prepared in media made by the above methods is of considerable value for immunising purposes. The data at present available, however, tend to show that it is of more value for subsequent immunisations than for preliminary immunisations.

The medium is of value for ordinary routine purposes, and would appear to afford an efficient, inexpensive, and readily prepared substitute for ordinary "peptone" medium.

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