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Production of Potent Diphtherial Toxin on a Medium of Chemically Defined Composition.

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As a result of studies carried out by one of us¹ on the chemical nature of the substances required by the bacillus of diphtheria for growth, and by another of us² on the factors underlying toxin-production by this organism, it is now possible for the first time to produce toxin of high titer on a medium of known composition. A qualification of the word "known" is admitted in that certain of the components are obtained from natural sources and may contain traces of impurity, and further, that the extremely important traces of certain inorganic elements are beyond control due to the effect of components from the type of glass containers used. The complete absence of peptone, formerly believed to be the *sine qua non* for toxin-formation, appears to be assured.

The composition and method of preparation of the medium is as follows:

glycine	0.5 g.	beta alanine	1 mg.
dl-valine	1.0 "	nicotinic acid	2 "
dl-leucine	0.5 "	K ₂ HPO ₄	2 g.
d-glutamic acid		NaCl	5 "
hydrochloride	5.0 "	MgSO ₄ · 7H ₂ O	0.3 "
l-cystine	200 mg.	CuSO ₄ · 5H ₂ O	5 mg.
dl-methionine	200 "	sugar lactate solution	
l-tryptophane	100 "	containing calcium	20 cc.
l-tyrosine	100 "	distilled water to	1000 "
pimelic acid	1 "		

The glycine, valine, leucine, glutamic acid, methionine and tyrosine, sodium chloride and potassium phosphate are weighed out and dissolved in about 500 cc. of distilled water. The cystine is dissolved in a minimal amount of 1:1 HCl and added to the main lot. The pimelic acid, beta alanine and nicotinic acid are now added and the solution adjusted to pH 7.8; 0.3 cc. of a 33% solution of calcium chloride is added and the medium boiled gently for 10 minutes. The

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¹ Mueller, J. H., *J. Bact.*, 1935, **30**, 513; *J. Bact.*, 1937, in press.

² Pappenheimer, A. M., Jr., and Johnson, S. J., *Brit. J. Exp. Path.*, 1936, **17**, 335.

precipitated calcium phosphate which carries down any inhibitory excess of iron is now filtered off. Finally the magnesium sulphate, copper sulphate and tryptophane are added and the medium made up to 1000 cc., dispensed and autoclaved. Before inoculation, 2 cc. of a sterile solution containing 37% Merck's U.S.P. sodium lactate, 7.5% Merck's c.p. glucose, 15% purified maltose and 0.3% calcium chloride are added per 100 cc. of medium.

This medium contains approximately 0.7 mg. of nitrogen per cc. The only iron present is that added as impurity in the sugar-lactate solution which fortuitously contains close to the optimal amount of that element.

Toxin-production of 2 Park 8 strains is summarized in Table I.

TABLE I.
Toxin-Production at 35°C.

Strain	Type of flask	Quantity of Medium cc.	Incubation (days)	Lf	L+	M.L.D.
Park No. 8 (Toronto)	Soft glass Fernbach	600	6	36	0.05	.00075
	Pyrex " "	600	6	18	—	—
Park No. 8 (Albany No. 5)	125 cc. Pyrex Erlenmeyer	25	4	18	—	.0015
	Soft glass Fernbach	600	7	24	—	.001
	125 cc. Pyrex Erlenmeyer	25	3	13	—	.0025

From Table I it is seen that the Toronto strains produced 36 Lf and 1300 M.L.D. per cc. on a known medium containing only 0.7 mg. of nitrogen per cc. It is noteworthy that this Lf value per milligram of nitrogen in the medium is at least twice that of the most potent toxin hitherto reported.³ The Albany No. 5 strain produced a somewhat lower titer than the Toronto but growth was less abundant than with the latter strain. It is possible that even higher titers of toxin will be obtained when studies now in progress on additional substances in the tissue-extract fraction as well as on the inorganic elements of the medium have been completed.

³ Taylor, E. M., *Ann. Inst. Pasteur*, 1935, **55**, 474.