9367 P

Substitution of β-Alanine, Nicotinic Acid, and Pimelic Acid for Meat Extract in Growth of Diphtheria Bacillus.

J. HOWARD MUELLER.

From the Department of Bacteriology and Immunology, Harvard Medical School, Boston, Mass.

In the course of studies extending over several years, the attempt has been made to define chemically the requirements of certain strains of diphtheria bacilli, which would permit growth equal or superior to that occurring on the usual peptone-infusion broth. This has been accomplished, as regards the peptone portion, for some of the strains, by means of amino acids[1] and certain differences and similarities between strains have been brought out.

An investigation of the extractive portion of the medium, absolutely essential for growth with all our strains, has led to the recognition that at least 3 substances are required by 2 strains. These are β-alanine, nicotinic acid, and pimelic acid. For a third strain the pimelic acid can be omitted. Omission of either β-alanine or nicotinic acid reduces the amount of growth practically to nil in the case of all 3 strains.

The quantities required to produce maximal effect are of the order of one microgram per cc. for β-alanine and nicotinic acid, and about 0.025 microgram per cc. for pimelic acid.

Pimelic acid apparently has not before been encountered in biological systems. The presence in liver-extracts of an ether-soluble acid-substance, from which a methyl ester could be prepared and distilled either *in vacuo* or at atmospheric pressure was first established. A better source of material having the same growth-stimulating and chemical properties was found in cows' urine. From 100 gallons of this material 0.6 g. of crystals proving to be pimelic acid were obtained. Details of this work are now in press.[2]

From a liver-extract concentrate representing 300 kg. of liver, about 10 mg. of crystals showing the desired growth-promoting property were obtained, and identified as nicotinic acid. Identification was facilitated by knowledge of Knight's[3] recent findings in connection with the effect of this substance on growth of the staphylococcus.

The third factor, β-alanine, has not actually been isolated from

1 Mueller, J. H., *J. Bact.*, 1935, **29**, 515; Mueller, J. H., and Kapnick, I., *J. Bact.*, 1935, **30**, 525.

2 Mueller, J. H., *J. Bact.* and *J. Biol. Chem.* In press.

3 Knight, B. C. J. G., *Nature*, 1937, **139**, 628.

liver-extract. The fraction containing the activity appeared to possess both COOH and NH_2 groups in equivalent proportions. Since a complete casein-hydrolysate was used in the control medium, β-alanine suggested itself as a possible component of the mixture and trial showed it to have practically the full effect of the liver-extract fraction. This compound, also, has previously been shown by Williams[4] to be of importance in the growth of certain yeasts, functioning as a part of the "bios" complex.

It is probable that other substances present in tissue-extract will increase somewhat the amount of growth of our test organisms. Thus, with the use of a complete tissue-extract, from 3.5 to 4.0 mg. of bacterial nitrogen are produced on 10 cc. of medium in 3 days' growth. On the same control base with the above 3 substances replacing the extractives, from 2.2 to 2.9 mg. of nitrogen are obtained. The usual peptone-infusion broth produces from 1.0 to 2.0 mg.

The following type-experiment illustrates the relative effect of the 3 substances on 3 strains of *C. diphtheriæ*—the National Institute of Health and the Alabama State Health Department's strains of the Park-Williams No. 8, and the "Allen" strain, isolated locally 2 years ago.

The relative amounts of growth are estimated by Arnolding 10 minutes, centrifuging and washing the organisms, and determining

TABLE I.

Composition of Medium		Mg. Bacterial Nitrogen N.I.H. Park 8	Ala. Park 8	"Allen" strain
1. Control* + ———		.07	.06	.08
2. " + pimelic acid 1γ (1)		(lost, very low)	.04	.10
3. " + nicotinic acid 10γ (2)		.02	.04	.21
4. " + β-alanine 10γ (3)		.10	.06	.08
5. " + (1) + (2)	amounts	.08	.00	.25
6. " + (1) + (3)	as	.08	.07	.10
7. " + (2) + (3)	above	.66	1.99	.64
8. " + (1) + (2) + (3)		2.32	1.86	2.24

*The control had the following composition:

Casein—HCl hydrolysate	.10	gm.
Cystine	.001	"
Glutamic acid	.050	"
Lactic acid (as Na salt)	.1	cc.
Salt mixture: NaCl	.050	gm.
$Na_2HPO_2 . 2H_2O$	.025	"
KH_2PO_4	.0035	"
$MgCl_2 . 6H_2O$	.003	"

In addition, the controls for the Park 8 strain contained 1.0 mg. tryptophane and 0.05 cc. ethyl alcohol. Quantities given are for 10 cc. of medium. The pH is adjusted to 7.4-7.6, the tubes autoclaved at 10 lbs. for 10 minutes, inoculated with a small loopful of pellicle from an 18 to 24-hour broth culture and incubated in a slanted position at 34°C. for about 60 hours.

[4] Williams, R. J., and Rohrman, E., *J. Am. Chem. Soc.*, 1936, **58**, 695.

the nitrogen of the sediment by microkjeldahl as previously described.[5]

Increasing slightly the concentrations of nicotinic acid and β-alanine improves the growth somewhat, but one or more additional substances evidently must be supplied for maximal growth.

Conclusions. Minute amounts of nicotinic acid, β-alanine, and pimelic acid, present together, permit about two-thirds the maximal growth of the diphtheria bacillus obtained from whole tissue extracts when added to a suitable control medium. The pimelic acid appears to be the least essential of the three. Differences appear between strains, even of the Park 8 organism, which may well be multiplied when further strains are examined.

9368

The Antigonadotropic Factor. Origin and Preparation.

BERNHARD ZONDEK AND FELIX SULMAN.

From the Laboratory of the Obstetrical-Gynecological Department, Rothschild-Hadassah Hospital, Jerusalem.

One of us[1] pointed out the fact that in rodents the gonadotropic effect diminishes if prolan from human pregnancy urine is applied in a protracted manner. Since that time the phenomenon has been repeatedly studied. Collip, Selye, *et al.*,[2] explained this as being due to the effect of antihormones, and succeeded in proving in the test-tube experiment the antisubstances against the hormones.

Proof of the presence of an antigonadotropic factor can be furnished only by the impairment of the gonadotropic reaction in rodents. As shown by our experiments[3] proof of the antigonadotropic factor by serological methods is not obtainable, pure prolan not forming any precipitins or complement-fixing antibodies. The sera of experimental animals which had been "immunized" with pure prolan over a period of more than 2 months do not show any serological reaction; they evince, however, a highly antigonadotropic effect.

---

5 Mueller, J. H., *J. Bact.*, 1935, **29**, 383.

1 Zondek, B., *Hormone d. Ovariums u. d. Hypophysenvorderlappens*, Berlin and Vienna, J. Springer, 1st ed., 1931, p. 159; 2nd ed., 1935, p. 272.

2 Collip, J. B., Selye, H., *et al.*, PROC. SOC. EXP. BIOL. AND MED., **1934, 31, 487,** 566, 1113; 1935, **32**, 544.

3 Sulman, F., *J. Exp. Med.*, 1937, **65**, 1.