

Amino Acids Required by the Diphtheria Bacillus for Growth.

J. HOWARD MUELLER.

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

The writer¹ described experiments indicating that a strain of diphtheria bacillus under investigation required for its growth, among other things, a substance associated with the "proline" fraction of amino acids obtained by Dakin's butyl alcohol extraction method. Further work with this material has shown that not a single factor, but 3 separate substances were involved in the accelerating effect there described. It was learned to begin with, that the action was not entirely limited to the "proline" fraction, but that the "monoamino" fraction even more frequently exerted it, and occasionally the 2 added together gave better results.

Before any material progress was made in separating and recognizing these factors, it became necessary to introduce a quantitative method for the estimation of relative amounts of growth. The method finally used was a micro kjeldahl determination of the amount of nitrogen in the bacterial growth on a measured amount of material for a definite time. The details of this procedure will be described elsewhere.

Careful examination of the effect produced by the proline fraction showed that proline itself was evidently not concerned, but that the acceleration in growth was apparently produced by minute quantities of ethyl alcohol used as solvent in handling the fraction. Pure ethyl alcohol exerted the same effect, and maximum acceleration of growth with our particular strain of organism is reached with a concentration of about 0.5%. Larger quantities of course become inhibitory. In place of ethyl alcohol, sodium acetate, glycerol, or maltose all appear to enhance the growth when added to the same control solutions.

The monoamino fraction, presumably containing glycine, alanine, valine, leucine, oxyproline, phenylalanine, tyrosine and methionine was next examined. Of these substances, only methionine showed any material acceleration of growth, when added to suitable control solutions. Its effect is striking in that small quantities, 0.25 to 0.75 mg. in 10 cc. of media approximately treble the growth on the con-

¹ Mueller, J. H., Klise, K. S., Porter, E. F., and Graybiel, A., *J. Bact.*, 1933, **25**, 509.

trol, whereas with slightly larger quantities, 2.0 to 3.0 mg., the growth begins to decrease and drops progressively as the amount of methionine is increased, so that with amounts of between 10 and 40 mg. in 10 cc., the growth is but little better than, but appears not to drop below that which occurs in the control.

Addition of the crude "monoamino" fraction to media containing sufficient methionine to throw the curve on the downward slope, which of course introduces still more methionine, will nevertheless sharply increase the growth obtained to about twice the maximum obtained with methionine alone. None of the other recognized constituents of the mixture, either alone or in combination will produce a similar effect. Attempts at chemical isolation of this third factor supplied the information that it was precipitable with Ag_2O and $\text{Ba}(\text{OH})_2$, and trial showed that histidine, which is not supposed to be present in the fraction under investigation, would practically duplicate the effect of the crude mixture. The explanation for the seemingly combined effect of methionine and histidine is not apparent, and may prove to be of interest in connection with methionine metabolism in the animal body.

In the meantime it was found that glutamic acid also increased markedly the amount of growth obtained in controls containing the crude monoamino fraction. The effect is not shared by aspartic acid nor asparagine under the conditions of the experiment, and the action differs from that of the other amino acids now shown to be necessary in that in each other case, maximum growth is reached with a concentration of about 1.0 mg. per 10 cc. of medium, while with glutamic acid the effect is slowly progressive, and the curve of growth still ascends gradually with 50 or 60 mg.

The medium as at present prepared, which gives maximal growth with our strain has the following composition:

	%
Liebig's extract	.75
Salt mixture*	
Cystine	.01
Tryptophane	.01
dl or l Methionine	.01
Histidine hydrochloride	.01
Glutamic acid hydrochloride	.5
Ethyl alcohol	.5
Phenol red	.001

*The salt mixture now used is prepared as follows:

NaCl	70.0 gm.
K_2HPO_4	2.5
CaCl_2	0.15
MgSO_4	0.15
FeCl_3	0.05
HCl (conc.)	0.5 cc.
H_2O to 350 cc.	

0.25 cc. of this solution is added to each 10 cc. of media.

The reaction is brought to pH 7.4-7.6 by addition of NaOH, the phenol red in the medium serving as the indicator, and autoclaved at 10 lb. for 10 minutes. Each tube is inoculated with a 3 mm. loopful of pellicle from a 24-hour broth culture and placed in a slanted position in the incubator.

On 10 cc. of such media approximately 1.9 mg. bacterial nitrogen is formed in 55-60 hours at 35°. The omission of either cystine, tryptophane or methionine causes a drop in nitrogen to the level of 0.2 to 0.3 mg. The growth obtained with this same strain on the usual meat infusion-peptone broth (10 cc.) gives 0.8-0.9 mg. nitrogen.

7662 C

A Renal Lesion Occurring in Rats Maintained at Low Environmental Temperatures.

EATON M. MACKAY, ERNEST M. HALL, AND FRANCIS M. SMITH.

From the Scripps Metabolic Clinic, La Jolla, California, and the Department of Pathology, University of Southern California, School of Medicine, Los Angeles.

During a study of the influence of environmental temperature upon the metabolic rate and certain organ weights of the albino rat a number of these animals were maintained at room temperature of 5° and 10°C. When they were killed it was noticed that the kidneys of many were swollen, soft and mottled in appearance. Fig 1. His-

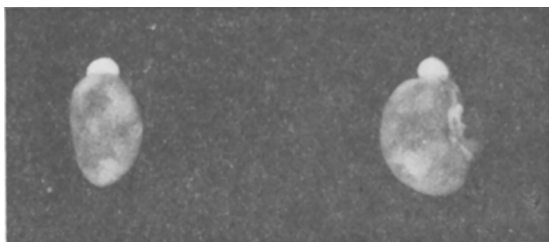


FIG. 1. Gross appearance of kidneys from rats Nos. 6 and 99, grown at 5° C.

tological examination revealed an interesting lesion in their kidneys. Besides its inherent interest it seemed desirable to report the occurrence of this lesion because of the common belief that exposure to cold is often associated with the onset of one form of Bright's disease in man. That the renal lesion reported here was acutally due to the cold environment seems certain for only 0.015% of the