adjusted to pH 6.5 with $M/15$ phosphate. A control digestion at the same pH was carried out with no activator present.

The results of this study, presented in Table 2, show the extent of stimulation realized by each of the treatments. With the pancreatic enzyme the inorganic salts increased the activity, but the increase seemed to be independent of concentration or kind of salt. A considerably greater increase in activity was observed when Roccal was used—about double the amount of activation afforded by the inorganic salts. Even with Roccal present, however, additional stimulation resulted from the addition of the sodium chloride. When calcium chloride and Roccal were both present, the activity was increased to 200% of the unstimulated digestion.

These data suggest a possible explanation of the growth stimulation obtained with chickens, rats, or pigs when they are fed detergents. It seems probable that the presence of the detergent permits a more ready penetration of the starch granule by the enzyme. This would make available a greater portion of the substrate for enzyme attack during the limited time in the digestive tract, and accordingly would increase the rate of starch digestion by the enzyme.

References

1. ELY, C. M. *Science*, **114**, 523 (1951).
2. HARRIS, R. S. *Arch Biochem. Biophys.*, **34**, 249 (1951).
3. LUECKE, R. W., HOEFER, J. A., and THORP, F., JR. *Quart. Bull. Mich. Agr. Expt. Sta.*, **34**, (3), 331 (1952).
4. STERN, J. R., GUTIERREZ, J. C., and MCGINNIS, J. Rept. Am. Chem. Soc. Mtg., Buffalo (1952).

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Putrescine as a Growth Requirement for *Neisseria*[1]

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Recently Herbst and Snell (*1*) have shown that the bacterium *Hemophilus parainfluenzae* requires the diamine putrescine for growth in a chemically defined medium. This was the first instance in which one of the putrefactive diaminic compounds was shown to be essential for bacterial growth. The compounds spermine, spermidine, and agmatine could replace putrescine, and it is conceivable that their activity is via putrescine, which would be formed as a result of hydrolytic cleavage.

Previous work in our laboratory, by Nemes *et al.* (*2*), on the nutritional requirements of nonpathogenic *Neisseria* has shown that many of these organisms require biotin in a vitamin-free casein hydrolysate medium. Attempts to cultivate *N. perflava* in media where the casein hydrolysate was replaced with complex amino acid mixtures were unsuccessful. Numerous vitamins and growth-factor compounds were then tested, and during the course of this work it was found that putrescine, in addition to biotin, was essential for growth of some strains of *N. perflava*.

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TABLE 1

COMPOSITION OF BASAL MEDIUM SUPPORTING GROWTH OF *N. perflava*

Amino acids	Amount/liter
DL-aspartic acid	1.0 (g)
L-glutamic acid	1.0
DL-alanine	1.0
L-arginine	0.20
DL-methionine	0.20
DL-threonine	0.20
DL-serine	0.20
DL-tryptophane	0.20
DL-valine	0.20
DL-phenylalanine	0.20
DL-isoleucine	0.20
L-lysine	0.20
L-leucine	0.10
L-proline	0.10
L-histidine	0.10
L-cystine	0.10
L-tyrosine	0.10
Glycine	0.10
Biotin	1.0 μg
Putrescine	1.0 mg
Salt solution A	5.0 ml
K_2HPO_4 25 (g)	
KH_2PO_4 25	
H_2O 250 ml	
Salt solution B	5.0 ml
NaCl 0.5 (g)	
$FeSO_4 \cdot 7H_2O$ 0.5	
$MnSO_4 \cdot 4H_2O$ 0.5	
$MgSO_4 \cdot 7H_2O$ 10.0	
H_2O 250 ml	

Employing the usual technical procedures for studies of bacterial growth in chemically defined media, it was found that the medium illustrated in Table 1 was capable of supporting growth of this organism on continued subculture.

The growth response of *N. perflava* (876) to graded amounts of putrescine is illustrated in Fig. 1. For this determination the medium was dispensed in 10 ml

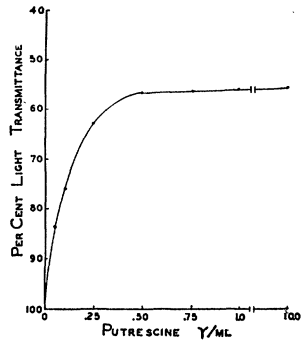

FIG. 1. Growth response of *N. perflava* to graded amounts of putrescine in a chemically defined medium.

amounts into 125-ml Erlenmeyer flasks and sterilized by autoclaving at 10 psi for 10 min. The inoculum consisted of approximately 0.01 ml of a washed cell suspension, and the incubation period was 48 hr at 35° C. Growth was measured turbidimetrically with a Klett-Summerson photoelectric colorimeter, using the blue filter. No growth occurred in the absence of putrescine, and maximum growth response was obtained at a concentration of approximately 0.5 μg/ml. Growth response of the organism was almost linear between 0.05 and 0.25 μg putrescine/ml. The presence of putrescine did not alter the biotin requirement for this organism. Three additional strains of *N. perflava* have been investigated, and all demonstrate a requirement for putrescine similar to that exhibited by culture #876.

Other diaminic compounds[2] which we have tested for growth response with *N. perflava* (876) indicate that spermidine, agmatine, and cadavarine may be substituted for putrescine. Of these compounds, cadavarine was the least active, and it is possible that its low activity might be due to contamination with putrescine.

Experiments are in progress at this time to determine the extent to which the amino acid content of the medium, as shown in Table 1, can be reduced without materially affecting growth, and to determine what effect these alterations may have on the vitamin requirements of this organism.

References

1. HERBST, E. J., and SNELL, E. E. *J. Bact.*, **58**, 379 (1949).
2. NEMES, J. L., PELCZAR, M. J., and DOETSCH, R. N. *J. Infectious Diseases*, **88**, 156 (1951).

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[2] We wish to thank E. E. Snell, Department of Biochemistry, University of Texas, for his kindness in supplying several of these compounds.

Deficiency of Ceruloplasmin in Patients with Hepatolenticular Degeneration (Wilson's Disease)[1]

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Wilson's disease (hepatolenticular degeneration) is associated with abnormalities in copper metabolism (*1*). The liver and the lenticular nucleus of the brain contain abnormally large amounts of copper (*2*), urinary excretion of copper is excessive (*1, 3*), the pigmented Kayser-Fleischer corneal ring, which is characteristic of the disease, presumably contains copper (*1*), and the level of serum copper has been reported to be abnormal (*4, 5*). Since most, if not all, of the copper normally present in serum is bound to ceruloplasmin (*6*), an investigation of this protein in Wilson's disease was undertaken. This paper reports the first results of the study, which show that there is a deficiency of ceruloplasmin in patients with this disease.

Ceruloplasmin is a blue α-globulin, with a molecular weight of 151,000, containing 0.34% copper, or 8 atoms copper/molecule (*7*). Normal plasma contains about 30 mg of the protein/100 ml so that ceruloplasmin constitutes roughly 0.5% of the plasma proteins. The copper appears to be an integral part of the molecule and is presumably responsible for its blue color, the absorption peak of which is at 6100 A.

Spectrophotometric. This method is based on the observation that the blue color of ceruloplasmin disappears on the addition of reducing reagents (*6*), such as ascorbic acid. Blood from fasting subjects was drawn, with precautions to avoid hemolysis. After centrifuging the blood twice and clarifying the plasma by Seitz filtration, light absorption was measured in a Beckman spectrophotometer from 5400 to 6600 A. A cell with a 5.0 cm path length was required. When successive measurements of the spectral curve showed no change with time, a solution of buffered sodium ascorbate was added in sufficient amount to make the plasma concentration 0.27%. Spectra were measured hourly for at least 5 hr at room temperature, by which time (although not much sooner), the maximum decrease in optical density at 6100 A had occurred. The concentration of ceruloplasmin was estimated by dividing this decrease in optical density, corrected for dilution, by the extinction coefficient $\varepsilon^{5\text{ cm}}_{1\%,\ 6100\ \text{A}} = 3.4$.

Immunochemical. Rabbits were immunized with either crystallized or purified ceruloplasmin (prepared and kindly supplied by D. R. Kominz and J. L. Oncley, of the University Laboratory of Physical Chemistry). The antisera obtained, even when crystallized ceruloplasmin was the antigen, gave precipitin tests with other purified plasma proteins, as well as with ceruloplasmin. The antiserum could be rendered specific for ceruloplasmin by repeated absorption with the serum of a patient with Wilson's disease which was particularly low in ceruloplasmin content. The method for the quantitative determination of ceruloplasmin in serum was similar to methods previously published (*8*). Copper determinations were made according to the method of Cartwright, Jones, and Wintrobe (*9*).

The results are shown in Table 1. It is apparent that in each individual the immunochemical method yields consistently higher values for ceruloplasmin than the spectrophotometric method. This is true

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