contrast to those of Feldberg[8]; his method of studying the problem of acetylcholine formation is however inadequate and his figures therefore open to criticism. A full discussion will be presented in a forthcoming paper.

The presence of the choline acetylating enzyme system in the axon is additional evidence for the role of acetylcholine suggested in connection with the parallelism established between the voltage of the nerve action potential and choline esterase activity[9a] and with the evidence that energy-rich phosphate bonds are adequate to account for the electric energy released by the nerve action potential.[9b] It is consistent with the view that the primary event responsible for the alteration of the membrane during the passage of the impulse is the release and removal of acetylcholine and that the energy of phosphate bonds during the recovery is used for the synthesis of the acetylcholine removed during activity.

DAVID NACHMANSOHN
HEDDA M. JOHN

DEPARTMENT OF NEUROLOGY,
COLLEGE OF PHYSICIANS AND SURGEONS,
COLUMBIA UNIVERSITY

THE EFFECTS OF PYRIMIDINES ON THE GROWTH OF LACTOBACILLUS CASEI

INVESTIGATIONS in these laboratories concerning the specificity of the response of *Lactobacillus casei* to folic acid have led to a study of the effects on this organism of various pyrimidines. When *L. casei* is grown in a medium deficient in folic acid only, the organism can utilize thymine in lieu of folic acid.[1] However, with thymine as the nutrient, the acid production does not surpass a level approximating one half the maximum attainable with folic acid.[1, 2] Enterococci which require folic acid also respond to thymine, some strains with maximal growth, some with a response much like that of *L. casei*. The response of *S. faecalis* (*S. lactis R*) to pyrimidines other than thymine and to related compounds has been studied without the discovery of any substance which produces a marked effect.[1,3,4] Corresponding studies with *L. casei* seem not to have been carried out.

The experiments to be reported here were designed to test the effect of each substance, not only as a possible stimulator of growth, but also as a possible inhibitor of the growth elicited with thymine or folic acid as nutrilite. A medium of approximately the same composition as that of Landy and Dicken[5] was used. Each substance, in amounts ranging from 0.0005 to 1 mg, was added to 10 ml of (1) the basal medium, (2) medium containing thymine in an amount sufficient to produce the full response (10 γ) and (3) medium containing folic acid sufficient to give about half-maximal growth (0.001375 microgram unit).[6]

Among the more than one hundred substances tested, twenty or more were found to give a significant biological response of one kind or another. Some examples of the experimental results are given in Table 1.

TABLE 1

RESPONSE OF *L. casei* TO VARIOUS PYRIMIDINES

Substance	Concentration mg/10 ml	Effect of pyrimidine in various media Change of titre per cent.		
		Unsupplemented	With thymine*	With folic acid†
Thymine	0.01	+500	0	+20
5-Methyl cytosine	0.1	+200	. .	. .
5-Methyl isocytosine	0.2	+300	0	+10
5-Methyl-2,4-diamino-pyrimidine	1.0	+300	−20	+20
5-Methyl-2-thio-4-oxy-pyrimidine	0.05	− 30	+10	+10
5-Methyl-2,4-dithio-pyrimidine	0.25	+ 20	−25	0
5-Methyl-2-oxy-4-thio-pyrimidine	0.004	+300	+15	0
5-Methyl-2,4-dioxy-6-imino-pyrimidine	1.0	− 10	0	0
5,6-Dimethyl 2,4-dioxy-pyrimidine	1.0	0	0	0
2,5-Dimethyl-4-oxy-pyrimidine	1.0	+ 30	+10	+10
5-Hydroxy uracil	1.0	− 50	−70	−75
5-Amino uracil	1.0	− 50	−25	−55
5-Carbamido uracil	1.0	− 50	−55	0
5-Chloro uracil	1.0	+ 45	−93	+20
5-Bromo uracil	0.2	+ 20	−40	+15
5-Iodo uracil	1.0	0	−40	−20
5-Nitro uracil	0.2	0	−14	−65

* 10 micrograms per 10 ml.
† 0.001375 microgram equivalent per 10 ml.

In the series of compounds which may be regarded as derivatives of thymine in which one or both of the oxygens is replaced by an imino or thio group, many of the members simulate the biological action of thymine. The replacement of oxygen by the imino group appears in each instance to weaken the activity so that approximately ten times as much imino as oxy compound is required to produce a response of the same magnitude. Since the two monoimino compounds are equal in activity and the response of *L. casei* to mixtures of these compounds can be predicted as an additive function of the responses to the individual components, and since 5-methyl-isocytosine

[8] W. Feldberg, *Jour. Physiol.*, 101: 432, 1943.
[9] D. Nachmansohn, R. T. Cox, C. W. Coates and A. L. Machado, (a) *Jour. Neurophysiol.*, 5: 499, 1942, and (b) 6: 383, 1943.
[1] J. L. Stokes, *Jour. Bact.*, 48: 201, 1944.
[2] K. Krueger and W. H. Peterson, *Jour. Biol. Chem.*, 158: 145, 1945.
[3] H. K. Mitchell and R. J. Williams, *Jour. Am. Chem. Soc.*, 66: 271–274, 1944.
[4] T. D. Luckey, G. M. Briggs, Jr. and C. A. Elvehjem, *Jour. Biol. Chem.*, 152: 157, 1944.
[5] M. Landy and D. M. Dicken, *Jour. Lab. Clin. Med.*, 27: 1086–1092, 1942.
[6] As 7.7 per cent. concentrate obtained through the courtesy of Professor R. J. Williams.

produces consistently a slightly greater acid production than does thymine, it appears that the imino compounds act as such and not, as might have been supposed, primarily by deaminization to thymine. The action of the thio compounds is somewhat more complex. Thus, 2-thio- and 2,4-dithiothymine have only slight activity, whereas 4-thiothymine is approximately as active as thymine, perhaps due to the lability of the 4-thio atom.

The introduction of a substituent into the 6-position of the thymine molecule seems, in general, nearly to abolish the activity. Thus a second methyl group as in 2,4-dioxy-5,6-dimethyl or an amino group in the same position renders the compound essentially inactive.

As shown below, if the methyl group of thymine is replaced by an amino or hydroxy group, compounds with a considerable inhibitory activity are produced. The reverse change, replacement of the oxy group in the 2-position by a methyl group, might have been expected to produce a compound with similar properties. However, the compound formed, 2,5-dimethyl-4-oxypyrimidine, is essentially inactive. This is, perhaps, related to the fact that the oxygen atoms of thymine apparently exist in the doubly bonded lactam form.[7] The entrance of a methyl group in the 2-position, therefore, involves a considerable change in the configuration of the molecule, since it results in the elimination of one imide hydrogen atom.

Among the 5-substituted uracils, compounds which may be regarded as derived by the substitution of other groups for the methyl group of thymine, there were found a number of compounds which inhibit the growth of *L. casei*. Isobarbituric acid (5-hydroxyuracil) and 5-aminouracil have approximately the same molecular weight and would be expected to have a spatial configuration similar to that of thymine. Both strongly inhibit the growth of *L. casei*. Moreover, growth can be restored by increasing the concentration of either thymine or folic acid. It is reasonable to suppose, therefore, that these substances exert their action by the displacement of thymine from active enzyme centers or structural elements and suggest the possibility of a metabolic function of the methyl group which can not be performed by the hydroxy or amino groups. Since the effects of these two inhibitors are exerted with either nutrilite, these observations are not inconsistent with Stokes's view of thymine as a product of an enzyme system which involves folic acid as a prosthetic group or coenzyme.[1] That this hypothesis is not an adequate explanation of the action of thymine, however, is brought out by a consideration of the effects of the 5-halogenopyrimidines and of 5-nitrouracil. For example, bromouracil can inhibit completely the growth of *L. casei* with thymine as the nutrient but may have no effect or produce a slight stimulation when folic acid is used as the nutrient. It follows that the growth of *L. casei* with folic acid as nutrient does not depend on an intermediary synthesis of thymine as such. Moreover, nitrouracil can prevent the growth of *L. casei* with folic acid at concentrations which have little or no effect on the growth with thymine. These observations suggest that the two nutrients act, in some fashion, as alternatives rather than as two components of an anabolic system.

GEORGE H. HITCHINGS
ELVIRA A. FALCO
MARION B. SHERWOOD
THE WELLCOME RESEARCH LABORATORIES,
TUCKAHOE, N. Y.

[7] J. R. Loofbourow, Sr. Miriam Michael Stimson, O.P., and Sr. Mary Jane Hart, O.P., *Jour. Am. Chem. Soc.*, 65: 148, 1943.

THE THYMUS AND ACETYLCHOLINE SYNTHESIS

THE possible involvement of the thymus in myasthenia gravis has led to the suggestion by Trethewie and Wright[1] that the thymus may influence the formation of acetylcholine (Ach), and by McEachern[2] that it may produce a curare-like substance which interferes with the action of Ach. Experimental evidence concerning the thymus and Ach synthesis is conflicting. Stoerk and Morpeth[3] incubated minced rat brain in Locke solution, with and without added thymoma tissue from a patient who died from myasthenia gravis, and found no significant difference in the amount of Ach synthesized in a three-hour period. Trethewie and Wright,[1] in two experiments, obtained slightly greater yields of Ach when ground brain was incubated with infant thymus than in controls without thymus. In one experiment, using thymus tissue from a person with myasthenia gravis, they obtained a small decrease in Ach formation.[4]

Experiments testing a possible effect of newborn rat thymus on Ach synthesis by brain slices of adult and newborn rats will be reported briefly.

EXPERIMENTAL

In five experiments the brain of an adult rat was split sagittally and each half sliced thinly. The slices of each half were weighed and placed in Locke solution containing glucose 1:500 and eserine 1:5,000. Ground thymus glands from twelve rats less than 24 hours old were added to one lot of slices and both lots

[1] E. R. Trethewie and R. D. Wright, *Aust. and New Z. Jour. Surg.*, 13: 244, 1944.
[2] D. McEachern, *Medicine*, 22: 1, 1943.
[3] H. C. Stoerk and E. Morpeth, SCIENCE, 99: 496, 1944.
[4] See also Torda, C., and H. G. Wolff, *Proc. Soc. Exp. Biol. and Med.*, 57: 69, 1944 for the effect of ether extract of the thymus on synthesis of acetylcholine.