those here reported. Had it been practical to give penicillin before breakfast and take the necessary blood levels for two- and three-hour periods under fasting conditions, better results would presumably have been obtained. However, since the clinical use of oral penicillin requires administration one or two hours after eating as well as before meals, these findings are applicable to the actual conditions of treatment. To provide optimal conditions for the absorption of penicillin, by mouth, the patient should be instructed to avoid fat in the diet in order not to delay the emptying time of the stomach, and also to take the penicillin one hour before eating and two hours after eating.

SUMMARY

A convenient, practical and effective procedure of administering penicillin by mouth consists of mixing the drug with one tablespoonful of aluminum hydroxide. Substantially higher blood levels are obtained following a 50,000 and a 100,000 unit dose of penicillin with this method than after administration of the drug dissolved in water, either with or without previous ingestion of a mild antacid, such as aluminum hydroxide. Adsorption of penicillin on aluminum hydroxide appears to exert a local protective effect against that portion of gastric acid with which it comes into contact.

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ON THE FORMATION OF ACETYLCHOLINE IN THE NERVE AXON

THE high concentration of choline esterase in the axon and its exclusive localization at the neuronal surface is one of the essential facts in favor of the concept that the release and the removal of acetylcholine is an intracellular process generating flow of current, the action potential.[1] Nachmansohn and Rothenberg[2, 3] have recently shown that the esterase of the nerve axon is specific for acetylcholine: if the enzyme activity is tested on a number of esters, a typical pattern is obtained distinctly different from that of other unspecified esterases. This specific choline esterase is found, either exclusively or predominantly, in all nerve tissue whether taken from mammalian brain or electric organ of fish, from vertebrate or invertebrate, from gray matter containing cell bodies and synaptic regions or from axon only.

The presence of a specific and highly active enzyme mechanism for the removal of acetylcholine in the axon indicates that the ester is metabolized there at a high rate and suggests therefore the possibility that acetylcholine may be formed in the axon as well as at the synapse.

Direct evidence is now offered for the formation of acetylcholine in the axon. As shown by Nachmansohn and Machado[4] acetylcholine is synthesized by choline acetylase, an enzyme system extracted by them from brain but not found in other organs. This enzyme forms acetylcholine in cell-free extracts, in the presence of adenosinetriphosphate, under strictly anaerobic condition.[5, 6, 7] Choline acetylase has now been found in the axon as shown in Table 1. The

TABLE 1

FORMATION OF ACETYLCHOLINE IN THE NORMAL AND DEGENERATING SCIATIC OF RABBIT

	Normal			Degenerated	
Exp. number	wt used mg	ACh formed μg/g/hr	Time after section (hours)	wt used mg	ACh formed μg/g/hr
1	395	60.0	0	392	61.0
	322	59.0	(control)	328	50.0
2	380	19.0	64	620	4.9
3	558	20.0	72	564	2.6

methods used were the same as described previously. The sciatics of 6 rabbits were used for each experiment. The amount formed per gram per hour is about 50 μg as compared with about 100–150 μg in guinea pig brain. The amount of acetylcholine formed in the axon as compared with that at synaptic regions thus appears to have about the same ratio as previously found for choline esterase activity at these two places.

Following section of the nerve the enzyme activity decreases. Two days after section nearly the total initial activity is still found. At that period conduction is still possible. The observation is consistent with the concept that the release of acetylcholine is responsible for conduction of the impulse. Three days after section, when conductivity had disappeared, the enzyme activity had lost only a part of the initial value. The results and conclusions are in

[1] D. Nachmansohn in R. S. Harris and K. V. Thimann, *Vitamins and Hormones*, New York, 3, 1945. In press.
[2] D. Nachmansohn and M. A. Rothenberg, SCIENCE, 100: 454, 1944.
[3] *Idem, Jour. Biol. Chem.*, 158: 653, 1945.
[4] D. Nachmansohn and A. L. Machado, *Jour. Neurophysiol.*, 6: 397, 1943.
[5] D. Nachmansohn, H. M. John and H. Waelsch, *Jour. Biol. Chem.*, 150: 485, 1943.
[6] D. Nachmansohn and H. M. John, *Proc. Soc. Exp. Biol. and Med.*, 57: 361, 1944.
[7] *Idem, Jour. Biol. Chem.*, 158: 157, 1945.

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.

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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.