The current tests for penicillin sensitivity[8] do not take into account the inoculum size effects. The use of paper strip tests on heavily inoculated plates is likely to be misleading in cases of relatively sensitive strains producing penicillinase.

A simple test for penicillin resistance is proposed, which indicates the sensitivity of a staphylococcus culture, the presence and proportion of resistant individuals in it, and the degree of their resistance. The test also distinguishes resistant strains from relatively sensitive strains that produce penicillinase. This test may appear to be too delicate because of the fine differences in penicillin sensitivity which it reveals. It must be kept in mind, however, that concentrations of the order of 0.5 units per ml are almost the highest that can be obtained in the blood of patients. A reduction in blood level from 0.5 to 0.2 units per ml in the course of treatment may give a chance for growth to resistant bacterial cells present in a relatively sensitive strain. Moreover, the stepwise increase in bacterial resistance should lead us to consider any strain that is not very sensitive as a potential source of highly resistant variants. The therapeutic use of insufficient concentrations of penicillin may allow these variants to grow and spread, thus nullifying the usefulness of penicillin as a therapeutic agent.

Infections caused by penicillinase-producing strains consisting of individually sensitive cells may be susceptible to penicillin therapy if the number of bacteria present is small enough so that little penicillinase production occurs.

The use of similar tests for bacteria other than staphylococci should await more evidence regarding penicillin-fastness in those organisms.

Summary. The results of penicillin sensitivity tests for staphylococci are influenced by the size of the inoculum on two accounts: (a) the presence in sensitive strains of a minority of resistant individuals originating by mutation; and (b) the occurrence of penicillinase-producing strains whose cells are individually sensitive to penicillin. A simple laboratory test is proposed for the detection and quantitative measurement of penicillin resistance in staphylococcus.

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Effect of Anesthetics and Convulsants on Brain Acetylcholine Content.

J. M. TOBIAS, M. A. LIPTON, AND A. A. LEPINAT. (Introduced by R. W. Gerard.)

From the Department of Physiology, University of Chicago.

Whether acetylcholine plays an important role in central nervous system synaptic transmission has long been a matter of interest. It is known that central neural tissue contains the ester, can respond to its administration and can synthesize it. Though such facts, taken together with information on peripheral conduction, may suggest a transmitter role for acetylcholine in the central nervous system they do not prove it. Therefore, additional evidence has been sought, and one approach has been to attempt a correlation of acetylcholine content with activity. Results have been varied. Thus, stimulation of afferent nerves has been found to increase production or liberation of acetylcholine by brain,[1,2] but negative results have also been reported.[3,4] Direct faradization

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[3] Adam, H. M., McKail, R. A., Obrador, S., and Wilson, W. C., *J. Physiol.*, 1938, **93**, 45P.

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of the isolated spinal cord of the rabbit[5] and toad,[6] and injection of KCl into the perfused cat brain[7] have been shown to liberate the ester. It has been reported that prolonged strychnine convulsions elevate brain acetylcholine in the frog,[1,8] but that neither strychnine, metrazol nor insulin convulsions raise it in the rabbit.[9] In the rat, on the other hand, there are conflicting reports on the effect of hypoglycaemic convulsions on brain acetylcholine content.[1,10] The effect of light on retinal acetylcholine in the frog has also been studied, and again divergent results have been obtained.[11,12,13]

In a further attempt to correlate brain acetylcholine with activity, we have measured, under constant conditions, the effect of two anesthetics (diffuse diminution of activity) and two convulsants (diffuse increase of activity) on brain acetylcholine content in the rat and frog.

Method. Whole rat brain, including medulla, was placed in a weighed homogenizer tube (Potter-Elvehjem) containing 2 cc of ice cold, eserinized frog Ringer (NaCl 0.67 g, KCl 0.015 g, $CaCl_2$ 0.012 g, $NaHCO_3$ 0.015 g, eserine salicylate 10 mg, and water to 100 cc). The tube was reweighed and homogenization carried out in the original 2 cc from the fourth through the sixth minute after decapitation. After adding eserinized Ringer up to a total volume of 9 cc per gram of tissue, the whole was mixed for one minute. The brei was then divided into two parts. An aliquot of one was assayed for free acetylcholine after centrifugation from the ninth to the twelfth minute at 3000 r.p.m. The remaining brei, acidified to about pH 4 with 0.5 N HCl, was placed in a boiling water bath for 2 minutes. When cool it was neutralized to phenol red, centrifuged, and assayed for total acetylcholine. Frog brain was similarly treated in a smaller homogenizer and made up to a final dilution of 10 cc per gram of tissue. It was assayed without centrifugation for total acetylcholine only.

The assay object was the eserinized, frog, rectus abdominis muscle mounted in a 4 cc muscle chamber. Emphasis has been laid on timing, since we have found that large discrepancies can result from irregular timing.

As we were interested primarily in any measurable relationship between acetylcholine content and activity, animals given convulsants were decapitated as soon as convulsions began. Thus, artefacts due to the anoxia of tonic seizures were avoided.

Results. The data are shown in Tables I and II. On the average, free acetylcholine in rat brain was 67 and 35% higher after chloroform and nembutal anesthesia respectively than in the unanesthetized animal. Total acetylcholine was 35 and 30% higher than without anesthesia. In the frog, total brain acetylcholine was 20% elevated after nembutal. Neither free nor total acetylcholine was elevated in rats or frogs showing convulsive activity after picrotoxin or strychnine.

Our failure to find any effect of strychnine on frog brain acetylcholine conflicts with earlier findings of others[1,8] who have reported an increase. On the other hand, strychnine has been claimed both to have no effect on rabbit brain acetylcholine[1] and to lower it.[14] These conflicting data on the effects of strychnine may in part be the result of different degrees and duration of anoxia developed.[10]

The only data we have seen on the effect of anesthetics were incidentally reported as

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TABLE I.
Effect of Anesthetics and Convulsants on Brain Acetylcholine Content.*

Acetylcholine in whole brain brei, μg per gram.												
Rat										Frog		
Normal†		Chloroform		Nembutal		Picrotoxin		Strychnine		Normal	Nembutal	Strychnine
free	total	free	total	free	total	free	total	free	total	total	total	total
0.7	3.3	1.4	4.4							4.4	5.7	4.8
0.6	3.0	0.9	3.9							5.4	6.3	6.6
0.6	3.5	1.2	4.5							4.5	5.3	4.9
0.6	2.1	0.8	2.7			0.6	2.5			5.4	6.2	4.8
0.6		0.9								5.0	5.8	4.6
0.6		0.8										
0.7		0.9										
0.5		0.9										
0.5		0.9										
0.6	3.7			0.9	4.9							
0.7	3.7			0.9	5.2							
0.7	3.3			1.0	3.9							
1.0	2.9			1.3	3.6	0.9	2.2	1.0	2.5			
1.0	3.1			1.5	3.3	1.0	2.0	1.1	2.6			
1.0	2.4					1.0	2.2					
0.7	2.3					0.4	2.2					
0.6	2.4							0.5	2.4			
0.5	2.5							0.9	2.4			
0.6	2.8							0.5	2.6			
—	—							0.6	2.8			

* Data on experimental animals are tabulated opposite controls run simultaneously.
† Average normal values, rat: free 0.7 (S.E. 0.04); total 2.9 (S.E. 0.14).

TABLE II.
Effect of Anesthetics and Convulsants on Brain Acetylcholine Content.
Average Data and Statistical Significance.*

Drug		Animals	Avg acetylcholine content, γ/g			
			Free		Total	
			content	difference	content	difference
Chloroform	10-60 min. anesthesia	normal rats	0.6 ± 0.02		2.9 ± 0.31	
		anesthetized rats	1.0 ± 0.06	0.4 ± 0.07	3.9 ± 0.41	1.0 ± 0.51
Nembutal	30 mg/kg. 10-60 min. anesthesia	normal rats	0.8 ± 0.08		3.3 ± 0.15	
		anesthetized rats	1.1 ± 0.12	0.3 ± 0.15	4.2 ± 0.37	0.9 ± 0.40
Picrotoxin	20 mg/kg	normal rats	0.9 ± 0.09		2.6 ± 0.19	
		convulsant rats	0.8 ±0.12	0.1 ± 0.07	2.3 ± 0.09	0.3 ± 0.21
Strychnine	3 mg/kg	normal rats	0.7 ± 0.11		2.7 ± 0.13	
		convulsant rats	0.8 ± 0.11	0.1 ± 0.11	2.6 ± 0.07	0.1 ± 0.15
Nembutal	30 mg/kg	normal frogs	—	—	4.9 ± 0.21	
		anesthetized frogs	—	—	5.9 ± 0.18	1.0 ± 0.28
Strychnine	3 mg/kg	normal frogs	—	—	4.9 ± 0.21	
		convulsant frogs	—	—	5.1 ± 0.11	0.3 ± 0.27

* Statistical significance is indicated as ±S.E., not as ±P.E.

part of a different type of investigation.[10] Three nembutalized rats yielded an average value of 0.35 or 0.43 (depending upon method) micrograms of acetylcholine per gram of brain, whereas the figure for 7 normals was 0.25 or 0.35 μg per gram. Here the direction and magnitude of difference agree with our findings.

In general, our values for free acetylcholine in the rat brain are somewhat higher than those reported elsewhere.[10] Preliminary experiments lead us to believe that the difference is probably a result of more efficient extraction because of finer homogenization obtained with the Potter-Elvehjem homogenizer than with the sand-in-mortar type used by others.

It is not yet possible to define rigorously

the physiological meaning of these results. One might assume that chloroform and nembutal raised brain acetylcholine above a "normal" level, but it is equally likely that the values obtained during anesthesia more nearly approximate the "true" resting levels. Thus greater activity, whether that of the usual waking state or that of the more active, convulsive state, is paralleled by a lower brain acetylcholine content than is rest. Whether this reflects an increased rate of acetylcholine breakdown during activity cannot be said. The change observed might have been much greater if brain regions rather than whole brain had been assayed. As the experiments were done, a large change in a small region may have been masked by the diluent effect of a large volume of tissue in which acetylcholine content did not change.

It will be recalled that chloroform liberates free acetylcholine from bound precursor *in vitro*.[15] For this reason we also used the quite different type of anesthetic, nembutal. In two experiments *in vitro*, nembutal did not liberate free from bound acetylcholine in homogenized rat brain. For this reason, and because bound acetylcholine was actually increased more than the free after chloroform or nembutal, our *in vivo* results probably do not reflect simple liberation of free ester from bound precursor. It is always possible that acetylcholine may accumulate as a result of esterase inhibition. Certain depressants have been shown to have anticholinesterase activity,[2,16,17] but this is apparently not the case with chloroform or single large doses of barbiturate.[18]

Conclusions. 1. Both free and total acetylcholine content of the whole rat brain are higher after nembutal or chloroform anesthesia (diffuse diminution of activity) than in the unanesthetized animal. The free acetylcholine change is more convincing after chloroform, whereas the total acetylcholine change is more convincing after nembutal. The total acetylcholine content of the frog brain is similarly elevated after nembutal.

2. Neither free nor total acetylcholine content of rat brain differed significantly after the onset of strychnine or picrotoxin convulsions (diffuse increase of activity) from that found in quiet animals awake. Nor did strychnine alter total acetylcholine content of the frog brain.

3. With the methods used, normal whole rat brain has been found to contain about 0.7 μg of free and 2.9 μg of total acetylcholine per gram wet weight; frog brain about 4.9 μg of total acetylcholine per gram.

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Effect of Nutrition on Susceptibility of Mice to Pneumococcal Infection.

GEORGE H. HITCHINGS AND ELVIRA A. FALCO.

From the Wellcome Research Laboratories, Tuckahoe, N.Y.

Several workers have studied the effects of various diets on the susceptibility of mice and rats to induced pneumococcal infections.[1,2,3,4] For the most part, these studies have dealt with the effects of B vitamin deficiencies. The observed differences between control and experimental animals have been small, and not altogether consistent from laboratory to laboratory.

It has been found in these laboratories

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