cleavage of the fermentation *L. casei* factor giving a pteridine fraction and an aromatic amine by using sulfurous acid, which is a milder treatment than that with trichloracetic acid.

The vitamin B_c conjugate of Pfiffner and associates,[15] which is also obtained from yeast, has been shown to contain 7 molecules of glutamic acid. This evidence indicates that there may be at least 2 different conjugates in yeast. If there are 2 conjugates of folic acid present in factor R, it may be that there are 2 enzymes needed to split them, both of which are present in hog kidney and only one in rat and chick liver.

All conditions necessary for the optimum efficiency of the rat and chick liver enzyme systems may not have been met, so that complete liberation of folic acid from the conjugate was not obtained. Every effort was made to determine the optimum conditions for the enzyme systems involved. The proper concentrations of the enzymes and substrate were determined, as well as the optimum pH and temperature for the incubation.

Hog kidney may contain an enzyme system that can synthesize folic acid from simple precursors, whereas chick and rat liver may be devoid of such an enzyme. Many bacteria, yeasts, and molds are known to possess enzyme systems that are able to take the pyrimidine and thiazole fractions of thiamine and combine them into the vitamin.

Summary. The growth and hemoglobin responses obtained in chicks receiving a purified diet supplemented with factor R were found not to be due to the preformed folic acid present in factor R.

By means of chick liver, rat liver, and hog kidney incubation procedures the folic acid content of factor R was greatly increased. This indicated the presence of potential folic acid which did not stimulate the growth of *S. faecalis*, but which was available to the chick. The potential folic acid liberated by hog kidney enzymes was found to account for all of the responses obtained in the chick, whereas chick and rat liver enzymes were not as effective in liberating folic acid.

The most probable explanation for the discrepancy in the folic acid content of factor R obtained following hog kidney and rat or chick liver incubations is that factor R is a mixture of conjugates of folic acid rather than one conjugate.

The response to factor R was not affected by the presence of succinylsulfathiazole in the diet. Conjugated folic acid appeared, therefore, to be available as such to the chick, or was made available by metabolic processes.

[15] Pfiffner, J. J., Calkins, D. G., Bloom, E. S., and O'Dell, B. L., *J. Am. Chem. Soc.*, 1946, **68**, 1392.

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Nerve Conduction After Inactivation of Choline Esterase.*

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Gilman reported in February, 1946[1] that di-isopropyl fluorophosphate (DFP) could block conduction in a stretch of frog nerve dipped in it; but that conduction returned on lifting out the nerve. Despite this "reversibility" of the block, the choline esterase (Ch.E.) was left inactivated. Objections were raised to the conditions of these experiments (atypical action potentials, manner of obtaining reversal, low frequency of stimula-

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[1] *The Physics and Chemistry of Nerve Conduction*, New York Academy of Sciences Symposium Monograph, in press.

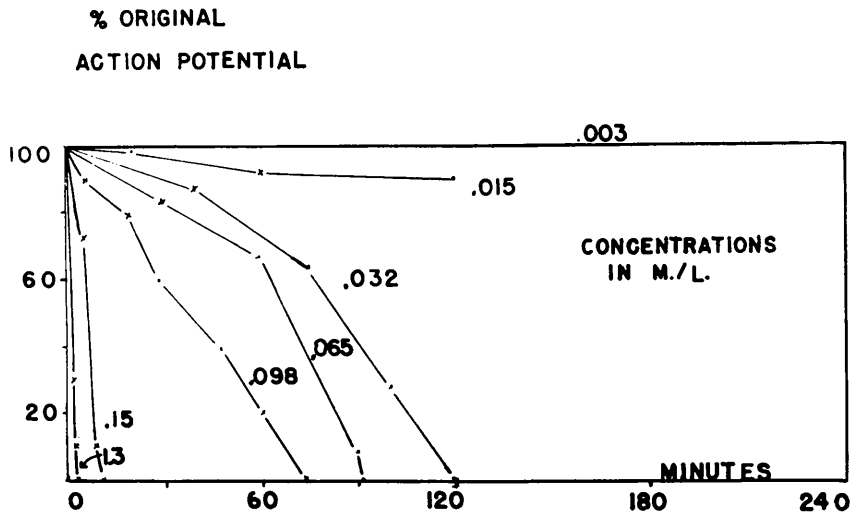

Fig. 1.

tion, small CO_2 values in the manometric determination of Ch.E., inactivation of Ch.E. at the time of grinding of the nerve, etc.).

A few weeks later, at the Federation symposium, succeeding papers by Crescitelli, Gilman, *et al.*[2,3] and by Rothberg, Nachmansohn, *et al.*[4,5] were the basis for an extensive further discussion. The former reaffirmed their findings, with added details; the latter reported that DFP depressed action and Ch.E. in parallel, and that both recovered in parallel on removing the drug, in squid nerve. The attention of other workers was invited to this experiment which, with other evidence (Gerard[1]), seemed potentially conclusive as to the role of A.Ch. in nerve conduction.

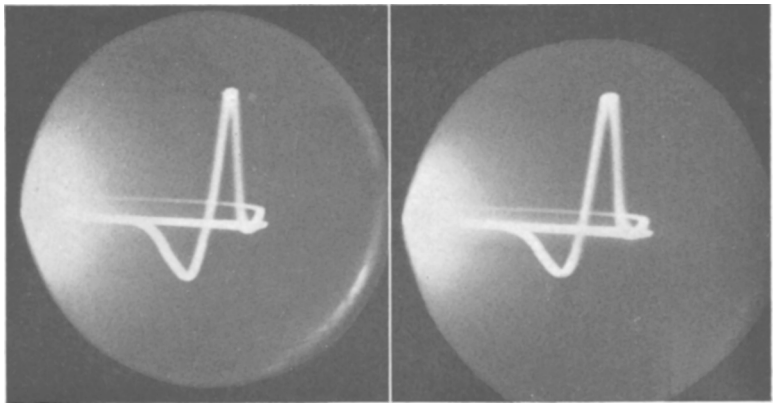

Fig. 2.
Left trace initial; right trace after 5 hours in 3 mM DFP. Read R to L. Conduction distance to first electrode 10 mm; to second one 30 mm. No crush, second electrode near cut end.

2 Crescitelli, F., Koelle, G. B., and Gilman, A. F., *Fed. Proc.*, 1946, Pt. II, **5**, 172.

3 Crescitelli, F., Koelle, G. B., and Gilman, A. F., *J. Neurophysiology*, 1946, **9**, 241.

4 Rothberg, M. A., and Nachmansohn, D., *Fed. Proc.*, 1946, Pt. II, **5**, 199.

5 Bullock, T. H., Grundfest, H., Nachmansohn, D., Rothberg, M. A., and Sterling, K., *J. Neurophysiology*, 1946, **5**, 253.

Method. We have modified the procedures as follows. Both sciatics of a frog were immersed in peanut oil, containing the desired concentration of DFP. The nerves rested on stimulating and lead-off electrodes and were tested in place. When desired, to test reversibility, the oil was well washed out of the chamber and fresh oil substituted. Normally, after 3 hours one nerve was removed from the DFP solution for Ch.E. assay, the other left in and its action potentials followed at intervals until the conclusion of the assay. Maximal stimuli (tested repeatedly during an experiment) were used at 20/sec. during the test.

To obviate any possible objection to the CO_2 method, Ch.E. was determined by a direct measure of the A.Ch. at appropriate times after mixing an A.Ch. solution with the homogenized nerve extract. Controls showed that peanut oil traces were without influence on rate of hydrolysis. A.Ch. was assayed on the frog's rectus with the usual precautions. Typical conditions were: 20 to 50 mg of nerve homogenized in ½ cc of Ringer, added to 4.5 cc of 4.5×10^{-4} M A.Ch. in Ringer, assayed after ½, 1, 2 and 3 hours at room temperature, approximately 20 C. At each time interval aliquots of the initial A.Ch. solution and of each of the nerve-A.Ch. suspensions were assayed for A.Ch. activity.† The initial solution as assayed was diluted to 0.5 γ A.Ch. Br. in 4 cc Ringer.

Results. In a preliminary series, nerves were followed in various concentrations of DFP until action potentials were abolished or no further change was observed in several hours. Fig. 1 shows a set of results. At DFP concentrations above 15 mM, the action potential progressively falls, during hours or minutes depending on the strength of the solution, to zero. Such a depressed or inactive nerve has never shown a sign of recovery when washed with fresh solvent. (On transfer from oil to Ringer return of potential occurred one time). At DFP concentrations below 15 mM, little depression of the action potential has been observed over a period of 2 hours; at 3 mM, none in over 6 hours.

In the critical experiments, DFP at concentrations of only 3 mM were used. Fig. 2 shows the practically unchanged action potential in a nerve left exposed to 3 mM for 5 hours while its fellow was being assayed for Ch.E. The latter, after 3 hours in the DFP, exhibited zero Ch.E. activity, as shown in Table I. That the enzyme was not inactivated only during homogenization, by DFP previously unable to penetrate, is shown by an experiment in which a DFP nerve and a control nerve were ground together (Table II). Ch.E. activity was more rather than less than the average of poisoned and unpoisoned.

TABLE I.
A.Ch. (as % of original amount) Present After 3 hr Incubation with Ground Nerve.

Exp.	Normal	DFP-treated
1	0	90
2	0	100
3	0	100
4	0	100
5	4	100
6	14	100
7	31	86

TABLE II.

Material tested (after 3 hr incubation)	Muscle response* (arbitrary units) Exp. A	Exp. B
Substrate alone	55	50
Substrate plus DFP-treated nerve	57	55
Substrate plus normal nerve	0	0
Substrate plus DFP and normal nerves	8	12

* Response is proportional to A.Ch. present.

Conclusions. Frog nerve, exposed to DFP in a concentration and for a time sufficient fully to inactivate its choline esterase, can still conduct impulses with entirely normal action potentials. Ch.E. is not essential to nerve conduction.

† Comparison of known and unknown solutions at each test is important, especially with DFP present. This substance, added with boiled or unboiled nerve but not by itself, potentiates the muscle response to known A.Ch. With 45 mM DFP, the muscle response may be doubled; at 6 mM the potentiation is around 20%; at 3 mM no potentiation was observed. The basis of this action has not been adequately studied, but no further formation of A.Ch. is involved.