enzyme. Moreover, it may be concluded that it is very improbable that any species of metallic ion is required for the catalytic function of acetylcholinesterase.

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On the Mg(II) Activation of Acetylcholinesterase[1]

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The activation of the enzyme acetylcholinesterase (AChE) by divalent cations has been studied by various investigators, using enzyme preparations derived from different animal sources. Nachmansohn[3] found that the activity of the enzyme obtained from the electric organ of Torpedo is quite markedly influenced by these cations, with decreasing power of ionic activation in the sequence $Mn^{++} > Mg^{++} > Ca^{++} > Ba^{++}$, and that a 2×10^{-5} M concentration of the most powerful cation Mn^{++} produced enhancement of catalytic activity by a factor of 14. More recently, van der Meer[4] observed that the activity of AChE from the red blood cell or that from the caudate nucleus of the rat is notably enhanced by the presence of Ca^{++} ion.

However, an interesting anomaly has been observed recently in experiments on Mg^{++} activation of purified[5] AChE from the same natural source, namely, the electric organ of the electric eel (*Electrophorus electricus, linnaeus*). Work in the Bethesda laboratories[6] pointed to a large decrease in activity of AChE functioning in a solution 0.01 M in Mg^{++} ion when complexing constituents such as citrate ion were present, at pH 7.4. In contrast, Wilson and Cabib[7] at Columbia University found little effect of these constituents on the enzymatic hydrolysis rates, and noted a maximum activating effect of Mg^{++} amounting to about a 20% increase in rate over that with no added Mg^{++} ion. A brief tabulation of the conflicting results for the two enzyme preparations, illustrated with data on both obtained in Bethesda, is presented in Table I. Data are given for the degree of activation produced by Mg^{++} at the 0.016 M level, the effect of added citrate at pH 7.4 on the enzymatic rates, and that of added Versene tested as a complexing agent at somewhat higher pH values. Enzyme purified at Bethesda is designated as enzyme A; that at Columbia University as enzyme B.

It is seen from Table I that, as noted in the Columbia experiments,[7] enzyme B is relatively insensitive to the presence of citrate ions or Versene, except for a small decrease in activity attributable to the complexing of a fraction of the Mg^{++} present, and is only activated by Mg^{++} at the 0.016 M level to the extent of about 30%. The increase in rate for experiment 4 as compared with experiment 2 can be ascribed at least in part to the increase in activity of AChE with increasing pH.[8] Enzyme A, on the other hand, demonstrates a much more marked sensitization by Mg^{++} ion, and desensitization of its catalytic power by agents that are effective in complex formation with this ion. Such behavior was found to be quite reproducible in repeated kinetic experiments made with fresh dilutions of a concentrated stock solution of enzyme A, and the stock solution was found to retain these properties completely on standing at 3–4°. Further, two different stock solutions of AChE prepared at Bethesda over a year's interval showed essentially the same Mg^{++} sensitization properties listed in the enzyme A column of Table I.

TABLE I

COMPARISON OF AChE PREPARATIONS

25.12°, substrate = $3.34 \times 10^{-3} M$, added NaCl = 0.081 M

Expt.	pH	Added Mg^{++}, M	Phosphate, M	Citrate, M	Versene, M	Relative activity: Enz. A[a]	Relative activity: Enz. B[a]
1	7.4	0.016	0.012			100	100
2	7.4	0	.012			15	71
3	7.4	.016		0.012		10	92
4	8.2	0	.011		0.014	18	85
5[b]	8.8	.014	.011		.028	8	

[a] Activity in phosphate buffer with 0.016 M Mg^{++} taken as reference value for each enzyme preparation. [b] NaCl concentration, 0.072 M.

The experimental cause for these differences between preparations A and B in Mg^{++} sensitization properties was brought to light by the exchange of diluted solutions of the two enzymes between the two laboratories. A solution of B which reached Bethesda after a day's travel at 3–4° was found to possess the properties in Table I previously noted by the Columbia group. However, a 1:25 dilution of stock enzyme A sent to Columbia, which had also aged at 3–4° for more than a day before being employed in kinetic experiments, was found to have *changed* its Mg^{++}—sensitization properties to those characteristic of enzyme B.

This phenomenon of change in susceptibility to Mg^{++} activation on aging in diluted solution, while the more concentrated protein solution retains its properties indefinitely, was checked in the Bethesda laboratories with the results shown in summary in Table II. Here, the same 1:25 dilution as that sent to Columbia was prepared, stored at 3–4°, and the activity of this dilution checked at approximately 20 and 60 hours after dilution under the conditions shown in Table II.

It is seen from Table II that the relatively high sensitivity to Mg^{++} activation of enzyme A, when tested immediately after dilution from stock, falls progressively on standing in *diluted* solution to the low sensitivity level characteristic[7] of enzyme B.

(1) The opinions in this paper are those of the authors and do not necessarily reflect the views of the Navy Department.

(2) Supported by a Grant from the Rockefeller Foundation.

(3) D. Nachmansohn, *Nature*, **145**, 513 (1940).

(4) C. van der Meer, *ibid.*, **171**, 78 (1953).

(5) Prepared essentially according to the procedures of M. A. Rothenberg and D. Nachmansohn, *J. Biol. Chem.*, **168**, 223 (1947).

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(7) I. B. Wilson and E. Cabib, *ibid.*, **76**, 5154 (1954).

(8) For the pH—activity curve of the enzyme, see the review by D. Nachmansohn and I. B. Wilson, *Advances in Enzymol.*, **12**, 259 (1951).

TABLE II

THE AGING OF A DILUTED SOLUTION OF ENZYME A

Dilution aged at 3–4°. In kinetic runs: temp., 25.12 ± 0.02°, [substrate]$_0$ = 3.34 × 10^{-3} M, [phosphate] = 0.012 M

pH	Added Mg^{++}, M	Versene, M	Activity after dilution 0 hr.	21 hr.	62 hr.
7.4	0.016		100	100	100[a]
7.4	0		15	50	76
8.2	0	0.014	18	79	91

[a] The absolute activity in this standard run with Mg^{++} present was found to have dropped only 1% from that observed at 0 hours.

It will be most interesting to note in further study whether any measurable change in state of the enzyme (molecular weight, ionic charge, light-scattering properties) accompanies this change in its response to Mg^{++} activation.

Experimental

The apparatus and techniques employed in the kinetic experiments were those previously described.[6,7] Recrystallized acetylcholine chloride as substrate and triply distilled water were used throughout.

The enzyme preparation B sent to Bethesda for the comparison of Table I was characterized by a specific activity (Ap value) of 6000 mg. acetylcholine hydrolyzed/hr./mg. of protein per ml. The Ap value of the stock solution of A was 11,000 and its content of ammonium sulfate was 5% by weight, with pH adjusted to 7. Results identical to those given for immediate dilution of stock enzyme A were obtained on a similar but older Bethesda preparation assayed at an Ap of 34,000.

In the aging experiments of Table II, the dilution of stock solution A (7.8 mg. protein per ml.) which was allowed to stand at 3–4° was a 1:25 dilution in water with a final ammonium sulfate content of 0.2%. A 1.0-ml. aliquot of the aging 1:25 dilution was made to 500 ml. with water just prior to kinetic experiments, and 0.50-ml. aliquots withdrawn for the individual runs.

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Cellulose Production by *Acetobacter xylinum* from Unlabeled Glucose and C^{14}-Acetate and C^{14}-Ethanol

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The significant enhancement of cellulose yield by *A. xylinum* brought about by the addition of ethanol to a medium otherwise suitable for pellicle production[1,2] is indicative that this 2-carbon alcohol can be an important ingredient in bacterial cellulose production. A similar response and effect have been observed when acetate is added to the same type of medium.[3] Despite the increased yield of bacterial cellulose from a glucose medium containing either one of these 2-carbon compounds, the organism is incapable of producing cellulose when either the ethanol or acetate is supplied as the sole carbon source.[1,2] The availability of labeled acetate and ethanol now makes it possible to determine how much, if any, carbon is furnished by these compounds for the biosynthesis of cellulose, thus permitting some insight as to the role played by these supplements as well as possibly elucidating upon the mechanism whereby the additional cellulose is produced.

In this note we wish to report the results of experiments in which *A. xylinum* was cultured in a glucose-enriched medium plus one of each of the following as the sole labeled ingredient: ethanol-1-C^{14}, ethanol-2-C^{14}, sodium acetate-1-C^{14} and sodium acetate-2-C^{14}. Analyses of the culture products are presented.

Experimental

Culture Conditions.—Except for the nutrient media, the methods of sterilization of medium, inoculation and incubation were the same as those used with D-glucose-1-C^{14}.[4] In this study the culture medium consisted of 1.0% unlabeled D-glucose; 0.3% KH_2PO_4; experiments 1 to 4 differed in the use of 0.5% Difco yeast extract, and experiments 5 to 7 differed in the use of 2.0% Difco yeast extract. Unlabeled sodium acetate, 0.6%, was added to experiments 3 and 4, and 0.1% to experiments 5 to 7, while none was added to experiments 1 and 2. Unlabeled ethanol, 0.76% was added to all experiments except 3 and 4. The total microcuries of C^{14}-labeled ethanol or sodium acetate added to each experiment appear in Table I.

Culture Products.—The C^{14} yields in the CO_2 formed by each culture were determined according to the procedures described earlier,[4] results appearing in Table I. The C^{14} contents of the celluloses (purified by the procedure described earlier[4]) were determined in two ways. The C^{14} in the celluloses from cultures 1 to 4, inclusive, was determined from a count of $BaCO_3$ obtained from the cellulose by a wet ashing procedure,[5] using Nuclear Instrument and Chemical Corporation's Q-gas Flow Counter, Model D-46A, in conjunction with Model 163 Scaler. Following the usual purification procedures the celluloses from cultures 5 to 7 inclusive were hydrolyzed, and the D-glucose purified by the method of Whistler and Durso[6] and by several recrystallizations before assay for C^{14}, using a vibrating reed electrometer which is more sensitive than the measuring instrument utilized in experiments 1 to 5, Table I.

Results

From the data presented in Table I it can be seen that little of the C^{14} originally furnished in the labeled acetate or ethanol was to be found in the resultant cellulose. The possibility that the traces of activity found in the cellulose from the earlier experiments (cultures 1–4 inclusive) represented non-cellulosic impurities was tested by using a more elaborate purification procedure and the vibrating reed as the measuring instrument in experiments 5,

TABLE I

Experiment no.	Labeled substrate	Total C^{14} in culture, microcuries	C^{14} found in CO_2, microcuries	C^{14} found in cellulose, microcuries
1	Ethanol-1-C^{14}	14	14	0.005
2	Ethanol-2-C^{14}	14	13.5	.04
3	Sodium acetate-1-C^{14}	19	14.6	.12
4	Sodium acetate-2-C^{14}	16	10.8	.18
5	Ethanol-1-C^{14}	33	21	None
6	Sodium acetate-1-C^{14}	46	36	.0003
7	Sodium acetate-2-C^{14}	79	68	.006

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