TABLE II

THE AGING OF A DILUTED SOLUTION OF ENZYME A

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

*p*H	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 *p*H 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 aliquots withdrawn for the individual runs.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 D46A, 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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(6) R. L. Whistler and D. F. Durso, THIS JOURNAL, **72**, 677 (1950).

6 and 7. The reduction in the proportion of radioactivity found in the cellulose from these cultures shows that impurities must have been present in experiments 1 to 4, and accounts for the higher value recorded.

As may be seen, the greater proportion of the C^{14} in each culture can be accounted for in the CO_2 evolved. The liquid culture products, exclusive of the CO_2 and cellulose, were radioactive and accounted for the remainder of the activity.

These observations indicate that, while both the ethanol and acetate are extensively metabolized by *A. xylinum*, neither of these 2-carbon compounds, nor any fragments thereof, appeared in the final cellulose product. It is interesting to note in this same respect the report[1] that this microorganism cannot synthesize cellulose from glycol, another 2-carbon compound. This information suggests that the use of any 2-carbon metabolic intermediate may be similarly restricted, and further investigation of the mechanism of cellulose synthesis by *A. xylinum* may well deal with this possibility.

The results of the present experiments indicate conclusively that even in the presence of D-glucose, neither ethanol nor acetate contribute carbon to the cellulose synthesis. The enhanced yields of cellulose are therefore caused in some other way.

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Regularities in the Basicity of Some Tertiary Ethylenediamines, Trimethylenediamines and 2-Hydroxytrimethylenediamines

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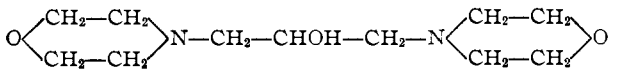
Pharmacological studies on certain tertiary amines called for an investigation of their basicities in aqueous solution. The amines were *sym*-disubstituted ethanes X-CH_2-CH_2-X (I), propanes X-CH_2-CH_2-CH_2-X (II), and 2-propanols X-CH_2-CHOH-CH_2-X (III), the substituents X being diethylamino (A), pyrrolidino (B), piperidino (C) or morpholino (D) groups linked to the ends of the carbon chain by their nitrogen atoms. These number and letter symbols will be used throughout the present paper; thus IIID is 1,3-dimorpholino-2-hydroxypropane



IA is tetraethylethylenediamine $(C_2H_5)_2N$-CH_2-CH_2-$N(C_2H_5)_2$, etc.

IA,[1] IIA,[2] IIIA,[3] IC,[4] IIC[5] and IIIC[6] are known compounds, the others are first described here. All are colorless or pale yellow oils of characteristic basic odor, with the exception of ID which is a solid, melting at 64°. Their corrected boiling points, determined at 760 ± 5 mm. pressure, are shown in Table I.

TABLE I

BOILING POINTS

	A	B	C	D
I	194	236	273	285
II	214	256	292	317
III	243	286	328	352

Table II shows the measured thermodynamic ionization constants as pK_A values; each column shows both the first and second ionization constants of the respective bases, and the difference Δ between the two ionization constants.

TABLE II

IONIZATION CONSTANTS

	A		B		C		D	
I	9.55	6.18	9.47	6.30	9.47	6.25	6.65	3.63
	(Δ = 3.37)		(Δ = 3.17)		(Δ = 3.22)		(Δ = 3.02)	
II	10.18	8.20	10.04	8.03	9.95	7.90	7.25	6.25
	(Δ = 1.98)		(Δ = 2.01)		(Δ = 2.05)		(Δ = 2.00)	
III	9.80	7.74	9.73	7.75	9.48	7.47	6.98	5.00
	(Δ = 2.06)		(Δ = 1.98)		(Δ = 2.01)		(Δ = 1.98)	

In each of the lettered series (A–D) the trimethylenediamine is the strongest base, the ethylenediamine the weakest, with the 2-hydroxytrimethylenediamine in between. The difference between the first and second ionization constants is uniformly close to two pK_A units in all bases which have a three-carbon chain between the two N atoms, and more than three pK_A units in the ethylenediamines. Similar differences (2.01 and 3.07 pK_A units, respectively) were found for unsubstituted trimethylenediamine and ethylenediamine, and interpreted in terms of induction and hydrogen bonding.[7]

Within each numbered series (I, II, III) there is little difference between the A, B and C compounds, but the D compounds are definitely weaker bases. This may be attributed to the inductive effect of the oxygen atom which reduces the basicity of the amino group much as it increases the acidity of a carboxyl group under similar circumstances (*cf.* pK_A of propionic acid, 4.89, and of lactic acid, 3.15). The same effect may be called upon to explain why series III is less basic than series II.

Experimental

The bases were prepared from the appropriate secondary amines and, respectively, ethylene bromide, trimethylene bromide and epichlorohydrin

$$4R_2NH + BrCH_2CH_2Br = 2R_2NH\cdot HBr + R_2NCH_2CH_2NR_2 \quad (I)$$

(1) A. W. Hofmann, "Jahresbericht ueber Forschritte der Chemie," 1861, p. 520.
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