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Note

Separation of choline and acetylcholine by cation-exchange chromatography

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Few, if any, simple chromatographic methods exist for a high-resolution separation of choline (Ch) and acetylcholine (ACh). Specifically, we were interested in a separation which met the requirements of the chromatography step of kinetic-isotope-effect measurements determined by the competitive, double-label technique[1,2]. These criteria are summarized below:

(i) Since the products and unreacted substrates of a reaction mixture are to be separated and analyzed, liquid chromatography, in which chromatographic fractions can be conveniently taken, should be used. This is in contrast to gas or thin-layer chromatography in which sample recovery may be difficult, result in large losses of material, and produce artifacts (as with scraping thin-layer plates) which interfere with subsequent analysis.

(ii) The separation of products and substrates must be *completely* resolved to a baseline established by a very sensitive technique, such as liquid scintillation counting.

(iii) The effluent must be easily removable from the chromatographic fractions containing products and unreacted substrates to yield pure materials free of solvents or buffer salts.

(iv) Conditions of the chromatography (*e.g.*, solvent, pH, duration of elution) must not cause the chemical decomposition or isotopic exchange of the compounds.

(v) The mode of chromatography and column dimensions must be compatible with a wide range of sample volumes, usually between 0.5 ml and 3 ml.

Although Gardiner and Whittaker[3] in 1954 reported a separation of Ch and ACh by cation-exchange chromatography, the resolution did not meet the above criteria. We decided to pursue the idea of separating Ch and ACh by cation-exchange chromatography and now wish to report a method which completely resolves these compounds and which meets our established criteria. Although this separation was designed to be used for competitive isotope effect determinations of acetylcholine hydrolysis, it should be applicable to a variety of experimental situations where a high resolution separation of Ch and ACh is indicated.

EXPERIMENTAL

Materials

The cation-exchange resin used in these experiments was Bio-Rad AG 50W-X4

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(NH_4^+) 200–400 mesh. [methyl-^{3}H]Choline chloride, [acetyl-1-^{14}C]acetylcholine iodide, Bray's solution (dioxane-based scintillation cocktail), and glass scintillation vials were obtained from New England Nuclear (Boston, MA, U.S.A.). Ammonium formate buffers were prepared by titrating NH_4OH solutions of known concentration with formic acid to the desired pH. Water was distilled from a copper-bottom still, deionized, and distilled from glass.

Scintillation counting of chromatographic fractions

Samples were prepared for liquid scintillation counting by adding to glass vials 1.0 ml of each fraction collected during a chromatographic separation and 10.0 ml of Bray's solution. These vials were dark-adapted for approximately 24 h and then counted in a Beckman LS200 liquid scintillation counter, each vial being counted for 5 min. Two channels of this instrument were used: counts in channel A correspond to those determined by use of the Tritium Iso-Set Module, while counts in channel B correspond to those determined by use of the Carbon-14 Iso-Set Module. Counting efficiencies and the channels ratio, cpm-B/cpm-A, were determined for [methyl-^{3}H]-choline and [acetyl-1-^{14}C]acetylcholine under counting conditions identical to those used throughout the chromatography studies and appear in Table I. Note that these were not rigorous determination of counting efficiencies, since such measurements are unnecessary here.

TABLE I

COUNTING EFFICIENCIES OF [^{3}H]Ch AND [^{14}C]ACh

Efficiencies were determined by counting a sample prepared by adding 1.0 ml of 0.2 *M* ammonium formate, pH 5.0 containing $5 \cdot 10^4$ dpm of either [methyl-^{3}H]choline or [acetyl-1-^{14}C]acetylcholine (dpm based on the addition of the appropriate volume of stock solutions of these compounds) to a glass scintillation vial. 10 ml Bray's solution was added to this.

Compound	*Channel efficiency*		*Channel Ratio B/A*
	A	*B*	
[^{3}H]Ch	0.13	0.00	0
[^{14}C]ACh	0.36	0.27	0.75

RESULTS AND DISCUSSION

A typical chromatogram depicted the separation of Ch and ACh appears in Fig. 1. This separation was achieved on a column of Bio-Rad AG 50W-X4 (NH_4^+), eluted with 0.2 *M* ammonium formate. The solution was buffered at a pH of 5 to eliminate effectively hydrolysis of ACh. It can be estimated[4] that at pH 5 less than 0.05% of the total ACh is hydrolyzed to choline and acetate in 15 h.

To determine the extent to which [^{3}H]Ch and [^{14}C]ACh were resolved by this chromatographic method, the channels ratio, cpm-A/cpm-B, was calculated for fractions having significant amounts of radioactivity. These results appear in Table II. The fractions containing [^{3}H]Ch have no [^{14}C]ACh, as indicated by the absence of counts in Channel B for these fraction. Furthermore, the fractions containing

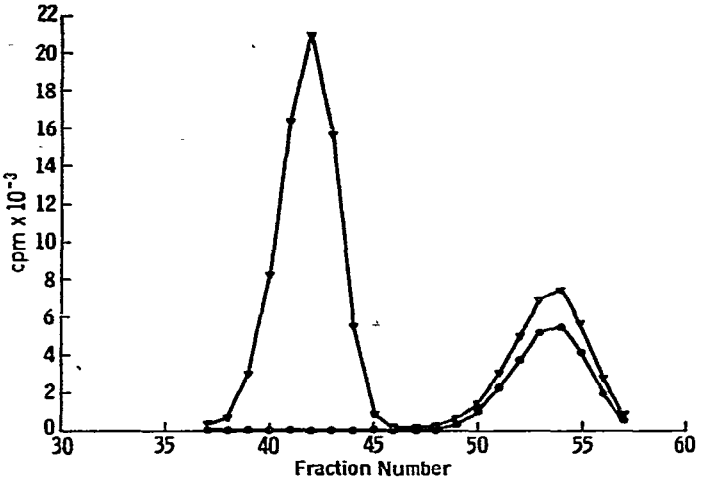

Fig. 1. Chromatography of [methyl-^{3}H]choline (fractions 37–46) and [acetyl-1-^{14}C]acetylcholine (fractions 49–57) on Bio-Rad AG 50W-X4 (NH_4^+). Column dimensions: 56 × 0.8 cm I.D. Elution with 0.2 *M* ammonium formate, pH 5.1 at a flow-rate of 47 ml/h (parastaltic pump used to achieve flow-rate). Fraction size was 10 ml. Sample applied to column contained 2 μCi of [^{3}H]Ch and 0.4 μCi of [^{14}C]ACh with total concentrations of Ch and ACh being 0.1 m*M* and 1.0 m*M*, respectively, in a total volume of 1.0 ml. Tritium (▲) and Carbon-14 (●) Iso-Set Modules were used for determining cpm.

[^{14}C]ACh have no [^{3}H]Ch contamination, as indicated by values of cpm-B/cpm-A which are identical within experimental error to the average value of 0.745 ± 0.010. Also, this average value of the channels ratio is identical to the channels ratio determined in control experiments (Table I) and the error associated with this average is the predicted value for samples containing between 5000 and 25,000 total counts in each channel.

In summary, then, a simple and general method has been developed for the

TABLE II

CHANNELS RATIOS FOR FRACTIONS COLLECTED DURING THE CHROMATOGRAPHY OF [^{3}H]Ch AND [^{14}C]ACh

Fraction No.	*cpm-A*	*cpm-B*	*cpm-B/cpm-A*	*cpm-B/cpm-A ave.*
39	2955	12	0.004	
40	8316	14	0.002	
41	16196	14	0.001	0.002 ± 0.001
42	21128	19	0.001	
43	15672	18	0.001	
44	5470	16	0.003	
50	1476	1082	0.733	
51	3024	2246	0.742	
52	4934	3760	0.762	0.745 ± 0.010
53	6892	5180	0.752	
54	7400	5484	0.741	
55	5710	4239	0.742	

column chromatographic separation of choline and acetylcholine using cation-exchange chromatography. This separation meets the criteria established for competitive determinations of isotope effects and other experiments requiring the complete resolution and convenient recovery of the Ch and ACh sample.

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