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Electrophoresis of acetylcholine, choline and related compounds*

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IN THE course of biochemical studies of compounds which act at cholinergic synapses we have found paper electrophoresis a considerably more satisfactory method for the separation of such bases than paper or ion-exchange chromatography,[1] fractional crystallization,[2, 3] gas chromatography,[4] techniques based upon solvent extractions,[5] or thin-layer chromatography on silica gel, cellulose, or alumina. The following results extend earlier reports by Chefurka and Smallman,[6] Henschler,[7] Frontali,[8] Ryall *et al.*,[9] and Friesen *et l.*[10, 11]

Chemicals were obtained from commercial sources except as follows. Acetyltriethylcholine was synthesized,[12] and β-methylcholine and thiocholine were prepared by alkaline hydrolysis of their acetyl-esters. ^{14}C-Choline (35 mc/m-mole) was obtained from Nuclear Chicago Corp. and ^{3}H-acetylcholine (45 mc/m-mole) and other isotopes from New England Nuclear Corp. Tritiated acetyltriethylcholine, acetyl-β-methylcholine, and acetylthiocholine (each 4·5 mc/m-mole) were prepared from ^{3}H-acetic anhydride; ^{14}C-benzoycholine was synthesized from benzoic acid.[13] Tritiated scopolamine (4100 mc/m-mole) was prepared by platinum-catalyzed exchange in $^{3}H_2O$.

Electrophoresis was performed at room temperature on 3 × 30·4-cm strips of Whatman 1 paper. Compounds were applied and air-dried on a pencil line drawn across the middle of the strips. Eight strips were mounted as 28-cm bridges in an inverted V-type Durrum cell (Beckman Instrument Co.) and were wetted with buffer to within 1 cm of the applied samples. The movement of fluid through the paper toward the samples then served to concentrate the materials to be separated, at the origin. After the strips had drained for several minutes, electrophoresis was carried out at a constant voltage of 500 (about 18 V/cm) for 1 hr. The strips were then left in a hood until they were barely damp. Separated substances were stained brown in iodine vapor and were outlined in pencil before the iodine evaporated; 0·1–1 μg of the compounds studied could be detected per cm^2 of damp paper. The position and recovery of labeled compounds on dried paper strips after electrophoresis were determined by counting 1·5-mm wide pieces of the paper in ethanol and a toluene-base phosphor by liquid scintillation spectrometry.

The most satisfactory buffer for our purposes was a 1·5 M acetic acid–0·75 M formic acid solution at pH 2; it gave the best distribution of isolated materials, was stable but completely volatile, and could be used to release organic bases from tissue fragments at the time of electrophoresis. The mobilities of 32 compounds in this buffer are given in Table 1, and some are illustrated in Fig. 1. The width of each applied sample was 2–3 mm, and the iodine stained bands are 3–4 mm wide. A mobility difference of 0·5 cm between two known compounds is therefore sufficient for their separate identification. Quaternary amines with more similar mobilities must be separated by longer runs. The mobilities of tertiary amines and of organic bases with carboxyl groups or other negatively charged centers may be altered at different pH values. In 0·1 M phosphate buffer at pH 8, for example,

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carnitine, acetylcarnitine, and betaine were almost immobile, and nicotine behaved as a mono-quaternary compound.

The resolution and recovery of the seven isotopic compounds was measured after electrophoresis of 1-μc samples of each with (n = 6) and without (n = 6) 10 μg of the corresponding non-radioactive

TABLE 1. ELECTROPHORETIC MOBILITIES OF ACETYLCHOLINE, CHOLINE, AND RELATED COMPOUNDS
Mobilities are given in cm/hr from the origin to the center of each band

Ethanolamine derivatives		Choline esters		Other compounds	
				Nicotine	12·0
				Hexamethonium	11·8
				Tetramethylammonium	10·6
				Succinylcholine	10·3
N-Methylethanolamine	9·8				
Choline	9·5				
β-Methylcholine	9·3				
Thiocholine	8·8				
				Decamethionium	8·7
		Acetylcholine	8·5		
		Acetyl-*β*-methylcholine	8·3		
		Carbamylcholine	8·2		
				Tetraethylammonium	8·2
		Propionylcholine	8·0		
		Acetylthiocholine	7·8		
N-Dimethylethanolamine	7·6				
Triethylcholine	7·5				
Carnitine	7·2				
		Butyrylcholine	7·2		
				Hemicholinium-3	7·0
				Pilocarpine	6·9
		Benzoylcholine	6·7		
		Acetylcarnitine	6·5		
		Acetyltriethylcholine	6·5		
				Neostigmine	5·9
				d-Tubocurarine	5·8
				Physostigmine	5·1
				Cocaine	5·1
				Atropine	4·9
				Scopolamine	4·8
				Betaine	4·0
				Acetyl-coenzyme A	0·5

compound. At least 96 per cent of each applied isotope was found in its stained band or the expected position of the band as measured from alternate strips; 99 per cent was recovered in the band and 1 mm of paper to each side of it. The resolution and recovery of choline and acetylcholine were not affected by spotting these substances with homogenates of 5 mg of rat brain or diaphragmatic muscle in 1 mM physostigmine. Under such conditions the separation of choline from acetylcholine was not less than 99·8 per cent complete. The resolution of these amines was also comparable when mixed with the usual biological salts, unless the capacity of the paper strips for cations (about 1 μmole-equivalent) was exceeded; band smearing then occurred.

Electrophoresis has numerous advantages over other methods for the isolation of organic bases. It is rapid and reproducible, gives very high and visible resolution, and is not affected by the presence of different anions or hygroscopic impurities which cause multiple-spot formation and tailing during paper chromatography. In addition, the movement of many substances can be predictably altered at different pH values to improve the degree of separation achievable.

The electrophoretic procedure described has permitted studies of the entry, turnover, and metobolism of labeled choline and acetylcholine in isolated nerve endings from brain, and in isolated rat superior cervical ganglia and hemidiaphragms. Separate papers on these results will be published.

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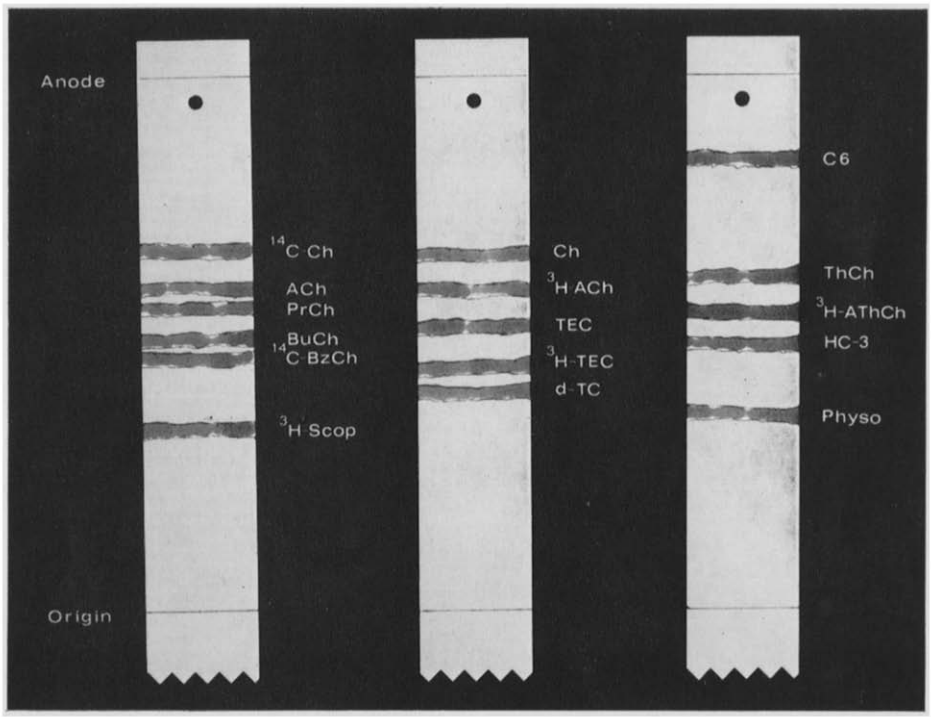

FIG. 1. Resolution of separate compounds by electrophoresis. Ten μg of each compound and 1 μc of several labeled substances were subjected to electrophoresis on Whatman I paper strips at pH 2 and 18 V/cm for 1 hr. The bands were stained in iodine vapor and outlined with a pencil before photography under fluorescent light. The contrast of the bands which is apparent on film exceeds many-fold the contrast visible by eye.

The following abbreviations are used. Ch, choline; TEC, triethylcholine; ThCh, thiocholine; ACh, acetylcholine; AThCh, acetylthiocholine; PrCh, propionylcholine; BuCh, butyrylcholine; BzCh, benzoylcholine; d-TC, *d*-tubocurarine; Scop, scopolamine; Physo, physostigmine; HC-3, hemicholinium-3; C6, hexamethonium.

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