

### Appearance of artifacts on chromatograms of quaternary ammonium compounds

In the course of extracting quaternary ammonium compounds from the electric organ of *Electrophorus electricus* (L.), aqueous extracts of the tissue were deproteinized with trichloroacetic acid and then extracted with ether. When the purified solutions were subsequently applied to the paper and the chromatograms run in alkaline solvents, it was found that the residual trichloroacetic acid caused the appearance of an artifact in the chromatographic pattern of the quaternary ammonium compounds present.

This alteration was observed in the case of choline and of several synthetic curares used in the study of the phenomenon of curarization in the eel. They were: 606 H.C.\* (hexamethylene-bis(carbamyl choline) diiodide), 336 H.C.\* (1,4-bis-(2-piperidinoethyl)-piperazine diethiodide), and F 2559 (Gallamine triethiodide, also known as Flaxedil).

Solutions of these compounds were prepared containing increasing amounts of trichloroacetic acid, then chromatograms were run in order to determine the amount necessary for appearance of the artifact.

The following alkaline solvent mixtures were used: ethanol-ammonia, propanol-ammonia-water, and butanol-pyridine-water.

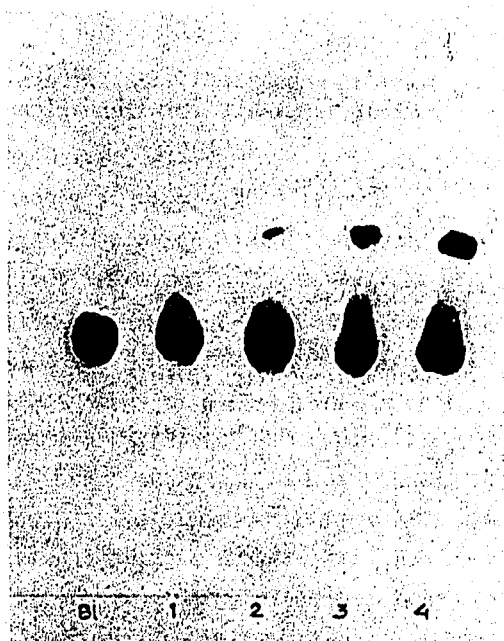


Fig. 1. Descending chromatogram run in ethanol-ammonia (95:5)<sup>4</sup>. Spot B1 contains 1  $\mu$ mole of choline chloride alone. The other spots contain the same amount of choline chloride to which increasing amounts of trichloroacetic acid had been added: spot 1 contains 0.2%, spot 2 0.3%, spot 3 0.4% and spot 4 0.5% trichloroacetic acid.  $R_F$  choline = 0.54;  $R_F$  artifact = 0.79.

\* Kindly supplied to Prof. CARLOS CHAGAS by Prof. JEAN CHEYMOL.

Reagents which are commonly employed to develop choline and curares on paper also reacted with the artifact. We used iodine vapours as well as reagents containing bismuth subnitrate<sup>1</sup>, bismuth carbonate<sup>2</sup>, chloroplatinic acid<sup>2</sup> and phosphomolybdic acid<sup>3</sup>.

The size of the artifact spot increased at the expense of the principal spot.

The synthetic curares 606 H.C. and 336 H.C. showed an additional spot in all three solvents when 0.2 to 0.4% trichloroacetic acid was present. At these concentrations of trichloroacetic acid, the artifact appeared in chromatograms of F 2559 run in butanol-pyridine-water and in propanol-ammonia, but not when ethanol-ammonia was the solvent, whereas in the case of choline chloride solutions, containing the same increasing amounts of trichloroacetic acid, the artifact appeared only when ethanol-ammonia was used.

When the artifact was eluted and rechromatographed in the same solvent, or in acid solvents (such as butanol-acetic acid or butanol-hydrochloric acid) it reappeared as such, and not as the parent quaternary ammonium compound.

Other authors<sup>5,6,7</sup> have reported trichloroacetic acid-produced artifacts in chromatograms of adrenaline, histamine and thyroxine when acid solvents were used, while we observed the phenomenon in the case of quaternary ammonium compounds when the solvents were alkaline.

This effect of trichloroacetic acid occurs frequently enough to suggest that care should be taken in the preparation and interpretation of chromatograms of substances extracted by procedures involving use of this agent, even when only very small amounts of it remain.

Thanks are due to Professor CARLOS CHAGAS for his helpful support.

*Institute of Biophysics, University of Brazil,  
Rio de Janeiro (Brazil)*

CHARITY CROCKER

<sup>1</sup> R. MUNIER AND M. MACHEBOEUF, *Bull. soc. chim. biol.*, 33 (1951) 846.

<sup>2</sup> C. CHAGAS, A. HASSON AND L. SOLLERO, *Hommage au Doyen René Fabre*, Sedes, Paris, 1956, p. 482.

<sup>3</sup> C. LEVINE AND E. CHARGAFF, *J. Biol. Chem.*, 192 (1951) 465.

<sup>4</sup> H. M. BREGOFF, E. ROBERTS AND C. C. DELWICKE, *J. Biol. Chem.*, 205 (1953) 565.

<sup>5</sup> D. M. SHEPHERD AND G. B. WEST, *Nature*, 169 (1952) 797.

<sup>6</sup> J. F. RILEY, *Nature*, 179 (1959) 882.

<sup>7</sup> J. D. ACLAND, *Nature*, 176 (1955) 946.

Received September 8th, 1958