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## Detection of choline-containing lipids on thin-layer chromatograms

At present, almost the only reagent used for the detection of choline-containing lipids on thin-layer chromatograms is the Dragendorff reagent<sup>1</sup>. However, this reagent possesses a number of drawbacks, especially low sensitivity and specificity<sup>2</sup>. The method suggested by SCHNEIDER<sup>2</sup> for the detection of choline derivatives on thin-layer chromatograms with phosphomolybdic acid was not widely applicable because of the painstaking procedure required and low specificity. BOTTCHEER AND VAN HONTE<sup>3</sup> suggested *cis*-aconite as reagent for the histochemical detection of choline; this reagent had previously been used for the quantitative determination of quaternary ammonium bases<sup>4</sup> and choline<sup>5</sup>.

We have used this reagent for the detection of choline-containing lipids on thin-layer chromatograms.

### Materials and methods

The preparation of the lipid extracts and individual phospholipids and the thin-layer chromatography were carried out as described earlier<sup>6,7</sup>. A 0.25 % solution of *cis*-aconite anhydride in acetic anhydride was used as reagent. The reagent was stable for a period of a week. After development in the solvent system, the plate was thoroughly dried at 110–115° for 10–15 min to remove traces of water. The hot plate was then sprayed with *cis*-aconite reagent and, after the development of red spots, the background was removed by spraying the plate with distilled water. The background disappeared and choline-containing lipids developed as bright red-violet spots.

### Results and discussion

When treating a plate with the reagent directly after development or after drying in air, the spots could not be distinguished from the background. This could probably be explained by the instability of the reagent towards water.

The minimum amount of lipid that could be detected was 1–2  $\mu\text{g}$ . Tests on individual lipids and mixtures of lipid from egg yolk, brain and sea invertebrates showed that the reagent detected only lipids that contained choline, while the Dragendorff reagent gave non-specific colouring of phosphatidyl-ethanolamine under the same conditions. The *cis*-aconite reagent is more sensitive than the Dragendorff reagent, and is very easy to prepare.

Although the results obtained with the *cis*-aconite reagent cannot be considered to be exhaustive, the reagent can be used for the detection of choline-containing lipids owing to its high specificity.

*Institute of Biologically Active Substances,  
Far-East Scientific Centre, Academy of Sciences of the U.S.S.R.,  
Vladivostok-22 (U.S.S.R.)*

V. E. VASKOVSKY  
Z. S. SUPPES

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