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## Note

# Complete separation of iodotyrosine–iodohistidine–iodide mixtures on thin-layer plates with a single-solvent system

WALTER M. GOLDBERG

*Department of Biological Sciences, Florida International University, Miami, Fla. 33199 (U.S.A.)*

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A large literature exists describing methods for the separation of iodo compounds. Iodotyrosines have been separated by thin-layer chromatography (TLC) and paper chromatography, ion-exchange column chromatography, gas–liquid chromatography, and Sephadex gel filtration[1–3]. However, the gel filtration technique recently described by Thomopoulos[4] is apparently the only method that has been described for the resolution of all iodotyrosines and iodohistidines. This paper describes a rapid and simple thin-layer system that separates all thyroidal iodoamino acids from one another and from iodide.

## MATERIALS AND METHODS

Plastic-backed Eastman-Kodak (Rochester, N.Y., U.S.A.) silica gel plates (No. 13179, without fluorescent indicator) were spotted with the following reagents: KI, 3-monoiodotyrosine ($T_1$), 3,5-diiodotyrosine ($T_2$), 3,3',5-triiodothyronine ($T_3$), and thyroxine ($T_4$) — all from Sigma (St. Louis, Mo., U.S.A.).

Monoiodohistidine ($H_1$) and diiodohistidine ($H_2$) were synthesized according to the method of Brunings[5]. All compounds were dissolved in 95% ethanol–1*N* HCl (2:1), except $T_4$, which was dissolved in acetone–1*N* HCl (1:1). The unactivated plates were placed in filter paper-lined 5-l TLC tanks which had been equilibrated overnight with benzyl alcohol–acetone–1*N* $NH_4OH$ (1:4:1). Chromatograms were run in the same dimension twice at room temperature over a distance of 15 cm. The total running time was approximately 7 h. Amino acid spots were visualized with ninhydrin, Pauly's or Folin's phenol reagent. Iodide was visualized with starch–iodate or ceric sulfate–arsenious acid–methylene blue reagent[6].

## RESULTS AND DISCUSSION

A typical chromatogram sprayed with ninhydrin and starch-iodate is shown in Fig. 1. Reproducibility is depicted in Table I. The benzyl alcohol solvent system is a modification of one of fifty given by Schorn and Winkler[3]. Fifteen of their systems were tried in all. Some of these separated the iodotyrosines in my hands while others did not. The benzyl alcohol system was the only one capable of separating all iodo compounds. It obviates the problems associated with two-dimensional systems[6] and

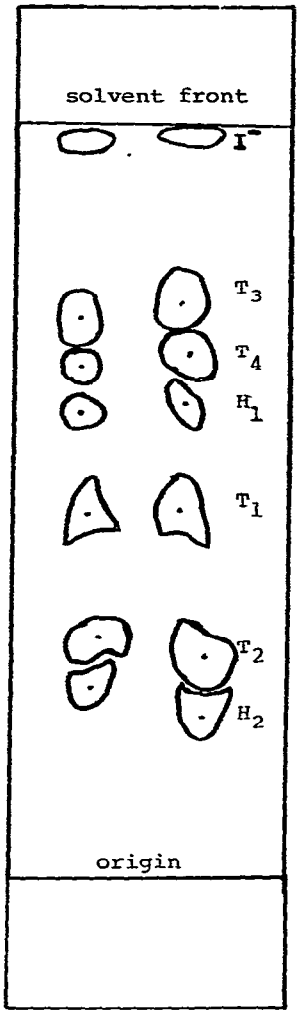


Fig. 1. Chromatogram trace showing the resolution of a mixture of iodotyrosines and iodohistidines on silica gel using benzyl alcohol–acetone–1 *N* $NH_4OH$ (1:4:1).

TABLE I

$R_F$ VALUES OF IODO COMPOUNDS WITH THE BENZYL ALCOHOL SYSTEM

| *Compound* | *$R_F$ range* | *$R_F$ mean* |
|---|---|---|
| $H_2$ | 0.21–0.23 | 0.22 |
| $T_2$ | 0.26–0.29 | 0.28 |
| $T_1$ | 0.42–0.49 | 0.46 |
| $H_1$ | 0.59–0.61 | 0.59 |
| $T_4$ | 0.63–0.68 | 0.66 |
| $T_3$ | 0.69–0.73 | 0.71 |
| $I^-$ | 0.92–0.99 | 0.95 |

mixed layer plates[7] that have been used to separate iodotyrosines in the absence of iodohistidines.

Modification of the system has been attempted. The ratios were permuted from 1:2:1 to 1:12:1; ammonium hydroxide was increased to 2*N* or substituted by acids; runs were made at varying temperature. No improvement was made by any of these alterations.

The method proposed here can be adapted to iodine metabolism studies where quick screening is required, or where the use of column chromatography is inappropriate.

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