Group separation of an aqueous solution of some iodinated amino acids and derivatives by means of solvent extraction

The serum-thyroxin extraction proposed in 1951 by MAN[1] goes back to the researches of LELAND AND FOSTER[2] and BLAU[3]. Since then, this procedure has been currently employed in the chromatography and quantitative determinations of thyroid hormones, and is based on the solubility of these substances in butanol and on their distribution coefficient with respect to a strongly basic aqueous phase. The main inconvenience of the employment of such a basic solution for the purification of the extract is the indiscriminate elimination from the butanol of some other iodinated substances of biological importance[4]. The formation of artifacts along the procedure is also worth while mentioning[5].

In this paper the solubility of different phenolic iodoamino acids and some deaminated derivatives is considered for a series of three aqueous–organic two-phase systems, in order to obtain an effective extraction of these substances from an aqueous mixture and a preliminary group separation which permits the ultimate chromatographic identification of the components of each group[6].

The following substances were tested: 3-monoiodo- and 3,5-diiodo-L-tyrosine; 3-monoiodo-, 3,5-diiodo-, 3,3',5-triiodo- and 3,3',5,5'-tetraiodo-DL-thyronine; 3,3',5-triiodothyroacetic-, 3,3',5-triiodothyropropionic-, 3,3',5,5'-tetraiodothyroacetic- and 3,3',5,5'-tetraiodothyropropionic acids (MIT, DIT, T_1, T_2, T_3, T_4, T_3A, T_3P, T_4A and T_4P, respectively), dissolved in 0.05 *N* NaOH (1 mg/ml). The solvents employed were *n*-butanol, chloroform and water at different pH's.

The distribution coefficients of these substances, in the chosen systems, were checked by shaking equal volumes of their solutions and the solvent in stoppered centrifuge tubes for 3 min. After separating the phases by centrifugation, the distribution of the substances in the systems and the formation of artifacts was checked by thin-layer chromatography of 10 μl aliquots of the phases investigated (corresponding to about 10 μg of substance). The phenolic group reaction of PAULY[7] and the iodinated compounds reaction of GMELIN AND VIRTANEN[8] permit the detection of 3 and 0.1 μg of substance, respectively.

The results given in Table I show that the separation of the deaminated thyroacids from the amino acids can be easily accomplished. The separation of T_3 and T_4 from the tyrosines, according to their different solubility coefficients, can also be achieved without difficulty. This is not the case concerning the separation of T_1 and T_2 from the tyrosines.

The following procedure, outlined in Table II, allows the proposed group separation, starting from an aqueous mixture of all the substances mentioned, 25 μg of each substance per ml in 0.05 *N* NaOH (Fraction 1). The extraction of 12 ml of the aqueous mixture with $^1/_2$ volume of chloroform after acidifying with a drop of conc. hydrochloric acid to pH < 2 permits the separation of the thyroacids which migrate quantitatively to the organic phase (Fraction 2), while the iodinated amino acids remain in the aqueous phase. On shaking this aqueous phase with $^1/_3$ volume of *n*-butanol, the thyronines and a part of the tyrosines pass into the butanolic phase, leaving in the water (Fraction 3) nearly half of the latter in a pure state. After extracting the butanolic phase with $^1/_2$ volume of 0.05 *N* NaOH (resulting pH $\geqslant 7$), half of the thyronines are isolated without impurities in butanol (Fraction 4) while the

TABLE I

DISTRIBUTION OF THE IODINATED PHENOLIC SUBSTANCES TESTED IN THREE DIFFERENT TWO-PHASE SYSTEMS

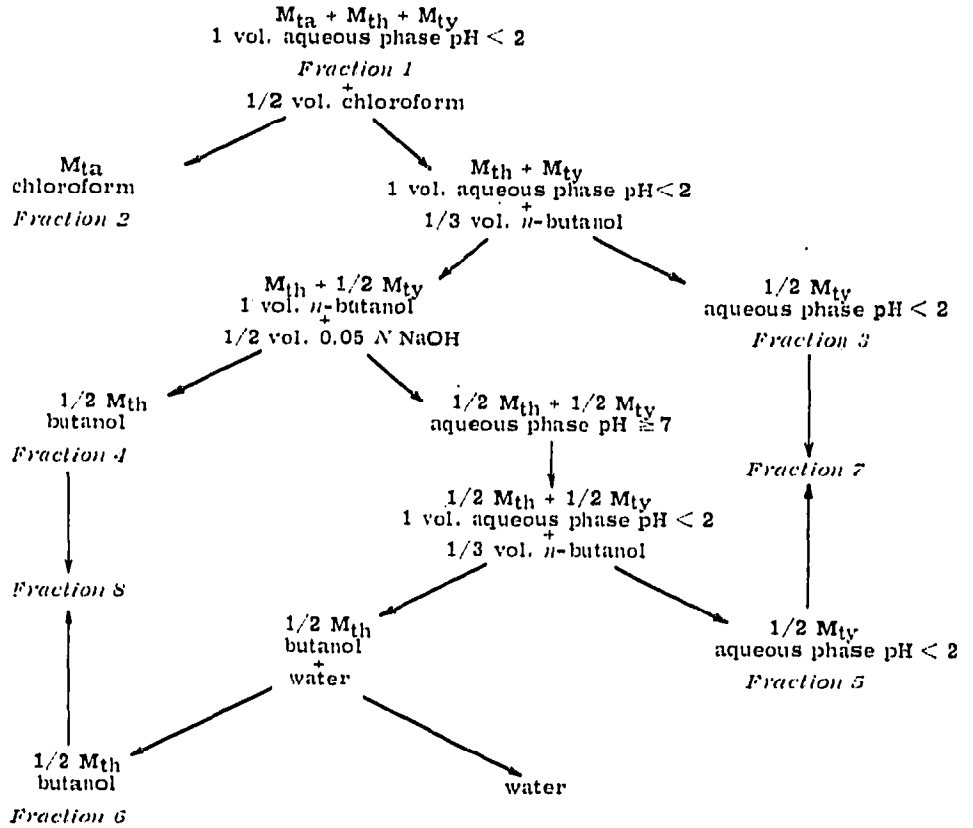
+ + + = all the substance in this aliquot (= 10 μg).
+ + = substance predominant in this aliquot (< 10 μg; > 3 μg).
+ = substance in small quantity in this aliquot (> 0.1 μg; < 3 μg).
— = no demonstrable substance in this aliquot (< 0.1 μg).

Substance	*Distribution between chloroform and water (pH < 2) (1st system)*		*Distribution between butanol and water (pH < 2) (2nd system)*		*Distribution between butanol and water (pH ⩾ 7) (3rd system)*	
MIT	—	+ + +	+	+ +	—	+ + +
DIT	—	+ + +	+	+ +	—	+ + +
T_1	—	+ + +	+ +	+	+	+ +
T_2	—	+ + +	+ +	+	+ +	+
T_3	—	+ + +	+ + +	—	+ + +	—
T_4	—	+ + +	+ + +	—	+ + +	—
T_3A	+ + +	—				
T_3P	+ + +	—				
T_4A	+ + +	—				
T_4P	+ + +	—				

TABLE II

EXTRACTION PROCEDURE FOR GROUP SEPARATION OF A MIXTURE OF IODOTYROSINES, IODOTHYRONINES AND DEAMINATED THYROACIDS

M_a = mixture of the four thyroacids; M_{th} = mixture of the four iodothyronines; M_{ty} = mixture of the two iodotyrosines.



rest of the iodinated substances migrate to the aqueous phase. If the recovery of these residual substances is desired, the aqueous phase is to be brought to the original volume of 12 ml with water and, after reacidification, is submitted to a fresh butanolic extraction. In this way the remaining iodotyrosines stay in the water (Fraction 5) and the iodothyronines pass to the organic phase. This phase (Fraction 6) is washed with $^1/_2$ volume of water in order to eliminate some impurities and mixed with the first butanolic extract, resulting in Fraction 8. The two aqueous fractions are also mixed to give Fraction 7.

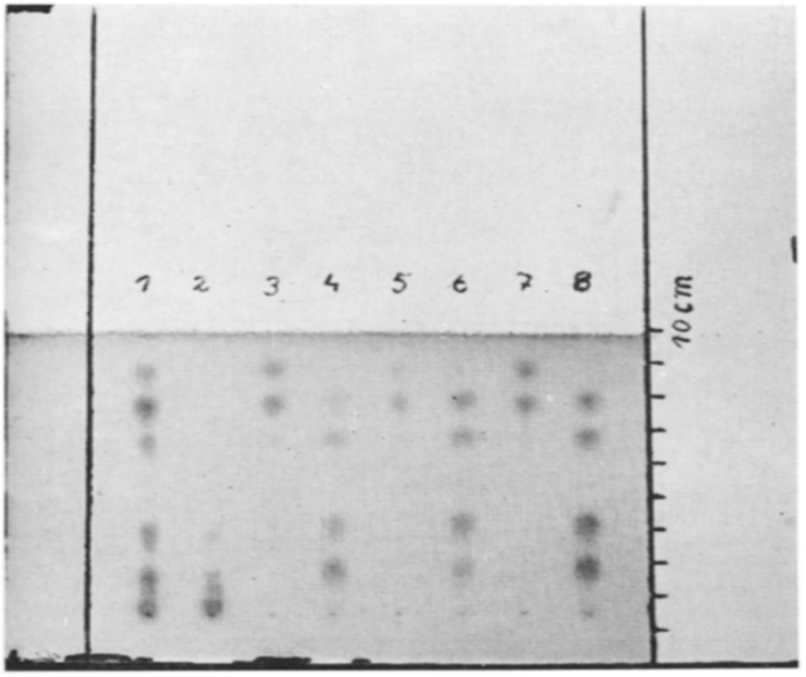


Fig. 1. Chromatograms of fractions 1–8. The spots in 1 correspond to the ten substances partially superposed. 2 shows the thyroacids poorly separated. In 3, 5 and 7 appear MIT (above) and DIT (below) and in 4, 6 and 8 (from top to bottom) T_1, T_2, T_3 and T_4. The chromatographic system consists of MN 300 G Macherey and Nagel as the stationary phase and acetone–0.5 *N* acetic acid (2:8) as the mobile phase[9].

Fig. 1 shows the chromatograms of all the eight fractions with equivalent volumes. The group separation attained is clearly seen and no artifacts are present.

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