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Deiodination of Thyroxine and Other Iodinated Compounds During Desalting by Electrodialysis.* (24184)

NICOLE ETLING AND S. B. BARKER

Department of Pharmacology, University of Albama Medical Center, Birmingham

Paper chromatography of thyroxine and its derivatives in a Ringer solution used for tissue metabolism studies is often unsatisfactory because the salts lead to training of the spots. Since desalting of solutions of ordinary amino acid can easily be accomplished using the electrolytic desalter of Consden, Gordon and Martin(1), an apparatus based on their design (Research Equipment Co.) was tried. The present study of rapid deiodination confirms and considerably extends the alterations in some iodinated compounds reported by Smith *et al.*(2) and by Jepson and Smith(3).

Methods. The desalter achieves results by electrolytic reduction of cations which are amalgamated at the cathode and passage of anions through a cellophane membrane into a flushing electrolyte solution. Conditions were chosen to remove completely the Na^+ and Cl^- from an isotonic NaCl solution, starting with 10 volts D.C. and 5-12 amps. The operation was finished in 10-15 minutes, as indicated by decrease in current to 0.2 amp. About 1 to 2 mg of iodinated substance† were dissolved in 0.05 ml N NaOH and diluted to 4 ml with 0.9% NaCl. Of this solution, 3.5 ml were treated in the chamber of the desalter. Aliquots representing before and after deionization were subjected to ascending chromatography, using n-butanol, ammonium hydroxide, water (250:72:178); n-butanol, glacial acetic acid, water (78:5:17); or methanol and 0.2 M ammonium acetate (100:250). The amino acids were revealed with ninhydrin(4, p. 88), phenolic substances with the Pauly reagent(4, p. 253) and iodine-containing compounds with ceric sulfate-arsenious acid(5).

Results. Thyronine, thyrosine and phenylalanine solutions all were desalted without loss of amino acids. The R_F values are shown in Table I. In contrast, mono- and diiodotyrosine were rapidly deiodinated to tyrosine. 3,3'-Diiodothyronine, 3,5,3'-triiodothyronine and thyroxine were deiodinated to thyronine, although in the last case, some free iodide remained in the cathode compartment. The iodine was also completely removed from 3,5-diiodothyronine, but some additional change was produced, since the product was clearly not thyronine. Further identification has not been possible, beyond demonstrating (Table I) that the material is a single phenolic amino acid (Pauly and ninhydrin-positive) not containing iodine. From the intensity of the ninhydrin and Pauly stains, it is probable that there was no loss of amino acid itself.

Table II shows the results of desalting car-

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TABLE I. Desalting of Derivatives of Thyroxine.

Original compound	R_F before desalting* 1	2	3	R_F after desalting* 1	2	3	Resulting compound
NaI		.11	.78				
Phenylalanine	.24	.28		.24	.27		
Tyrosine	.05	.14		.05	.14		
Thyronine	.34	.44		.34	.43		
3-Monoiodotyrosine		.28			.14		Tyrosine
3,5-Diiodotyrosine		.41			.13		"
3,3'-Diiodothyronine	.27	.62		.28	.43		Thyronine
3,5,3'-Triiodothyronine	.46	.63		.35	.41		"
Thyroxine	.29	.67	.16	.34	.41	.78	"
3,5-Diiodothyronine	.25	.34		.18	.23		Unknown

* Solvent systems: 1. Butanol-ammonia-water. 2. Butanol-acetic acid-water. 3. Methanol-ammonium acetate.

ried out on several thyroxine derivatives having fatty acid side chains in place of the alanine. Again, some deiodination was apparent, but specific identification of the products was not possible because information concerning the non-iodinated compounds was lacking. 3,5-Diiodothyroacetic and 3,5,3'-triiodothyroacetic acids were completely deiodinated to the same material, presumably 4-(4'-hydroxyphenoxy)-phenylacetic acid. The tetraiodothyroacetic acid was partially recovered unaltered. Since iodide was also encountered, some of the tetraiodothyroacetic must have been deiodinated to the iodine-free compound just mentioned, although not in large enough quantity to give a Pauly stain.

Tetraiodothyropropionic acid was only partly deiodinated, but 3,5,3'-triiodothyropropionic was completely stripped of iodine. With all 5 organic acid compounds, deiodination was sufficiently slow that traces of inorganic iodide could be detected in the solution with the extremely sensitive iodide test.

Tetraiodothyroacrylic acid proved to be far more stable than any other material, resisting alteration completely. In contrast, 3,5-diiodothyroacrylic and tetraiodothyroformic acids not only were completely deiodinated, but were both sufficiently altered that no Pauly reaction was obtained. This suggests that there may have been reduction of the hydroxyl or more drastic alteration of the ring structure.

Table III shows the effects of desalting sev-

TABLE II. Desalting of Fatty Acid Analogues of Thyroxine.

Original compound	R_F before desalting* 1	2	3	R_F after desalting* 1	2	3	Resulting compound
3,5-Diiodothyroacetic	.33	.88	.61	.33	.88	.44	Non-iodinated†
					.11	.77	NaI
3,5,3'-Triiodothyroacetic	.50	.88	.53	.33	.89	.44	Non-iodinated†
					.11	.79	NaI
Tetraiodothyroacetic	.47	.85	.45	.41	.86	.44	Tetraiodothyroacetic
					.11	.79	NaI
3,5,3'-Triiodothyropropionic	.57	.88	.53	.48	.87	.53	Non-iodinated†
					.11	.83	NaI
Tetraiodothyropropionic	.43	.85	.32	.41	.90		Non-iodinated†
					.11	.76	NaI
3,5-Diiodothyroacrylic	.67			—			None detectable
Tetraiodothyroacrylic	.46			.46			Tetraiodothyroacrylic
Tetraiodothyroformic	.53			—			None detectable

* Same solvent systems as Table I.

† The same non-iodinated, Pauly-positive compound in all 3 instances; probably desiodothyroacetic acid.

TABLE III. Desalting of Various Iodinated Compounds.

Original compound	R_F before desalting* 1	2	3	R_F after desalting* 1	2	3	Resulting compound
O-Methyl thyroxine	.53	.61		.42	.48		Deiodination
Thio-ether analogue of thyroxine	.35	.75		.33	.71		"
Thyroxamine	.85	.79		.90	.76		"
3′, 5′-Dichloro-3,5-diiodothyronine	.30	.73		.18	.48		"
3′, 5′-Dibromo-3,5-diiodothyronine	.19	.79		.25	.38		Deiodination (thyronine?)
2,6-Diiodophenoxyacetic					.10		NaI
3,5- "					.10		"
Tetraiodophenolphthalein	.56	.94		.55	.94		No change

* Same solvent systems as Table I.

eral other iodinated compounds, some derivatives of thyroxine and others quite different in structure. Since none of the deiodinated products is available, it is only possible to consider that loss of iodine and change of R_F demonstrate at least deiodination. Other alterations may be produced, similar to those found in 3,5-diiodothyronine (Table I).

O-Methyl thyroxine, the thio-ether analogue of thyroxine and thyroxamine were all deiodinated, the last much more slowly. Since 3′,5′-dichloro- and 3′,5′dibromo-3,5-diiodothyronines were deiodinated, it is probable that dehalogenation was complete, but the R_F's obtained after treatment are not truly the R_F of thyronine. Tetraiodophenolphthalein was completely stable. Two iodophenoxyacetic acids did not react with the ceric-arsenious acid reagent before treatment, but did show the presence of some inorganic iodide afterwards.

Conclusions. 1) Use of electrolytic desalter to remove inorganic ions from solution, results in removal of iodine from aromatic linkage, including thyroxine, triiodothyronine, diiodotyrosine and several of the fatty acid side-chain analogues of thyroxine. 2) This prevents use of apparatus for preparation of salt-free solutions in thyroid studies. The procedure might be useful in preparation of various dehalogenated derivatives where the parent compounds are readily available.

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Lactic Dehydrogenase Production in Tissue Culture of Normal, Transformed, and Malignant Human Cell Lines.*† (24185)

ALICE E. MOORE AND FELIX WROBLEWSKI

Divisions of Experimental Pathology and Enzyme and Metabolic Studies, Sloan-Kettering Institute for Cancer Research, Memorial Center, N. Y. City

The lactic dehydrogenase activity (LD) of serous effusions containing and/or bathing proliferating malignant neoplastic cells has been found greater than LD activity of blood plasma from which the serous effusion is derived. It was postulated that the greater enzyme activity might be due to its accumulation in serous fluid as a result of either multi-

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