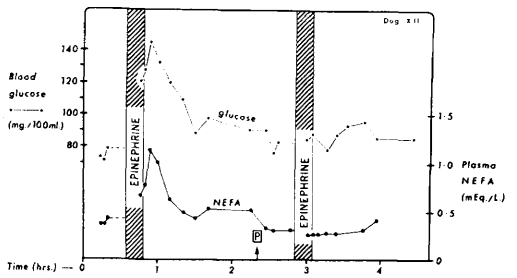

FIG. 4. Changes in blood glucose and plasma NEFA levels in response to epinephrine infusions. I.V. injection of propranolol is shown by the arrow.

Prior to blockade, epinephrine had little effect on arterial blood presure, but increased the cardiac output. Following blockade the arterial pressure was greatly increased and the heart rate and cardiac output decreased. This is explained by the fact that epinephrine is an agonist of both alpha and beta receptors. When the latter are blocked, activity at the former predominates, resulting in intense peripheral vasoconstriction, hypertension and slowing of the heart. That the slowing was reflexly induced was clear from the fact that it did not occur in atropinized dogs.

Release of NEFA from adipose tissue stores is to a large extent under the control of the sympathetic nervous system. Infusions of epinephrine(9) and norepinephrine(10) cause temporary increases in plasma NEFA levels. Most authors(11,12) found that pronethalol prevented the catecholamine-induced increase from occurring. However, Riggilo and Kyam(13) reported that, although pronethalol blocked the hyperglycemic effect of catecholamines in dogs, it failed to inhibit the increase in NEFA levels. In our studies, propranolol consistently and completely blocked the release of NEFA by epinephrine even when its hyperglycemic effect was not completely abolished.

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Separation and Quantitative Recovery of Iodinated Amino Acids and Iodide by Thin-Layer Chromatography.* (31567)

T. SOFIANIDES, C. R. MELONI, E. ALGER, AND J. J. CANARY
(Introduced by John C. Houck)
Division of Metabolic Disease and Endocrinology, Department of Medicine, Georgetown University Medical Center, Washington, D.C.

The physical separation of iodoamino acids has been studied in the past by means of paper and column chromatography and electrophoresis(1,2). Despite its reputation as a highly useful analytical tool in organic and biologic chemistry, few papers describing the separation of thyroid hormones by 2-dimensional TLC have appeared(3,4,5). In addition, the use of 2 solvents in the same direc-

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tion to effect separations has been described (6).

This communication describes a 2-dimensional method of TLC for use in the separation and quantitative recovery of radio labeled iodoamino acids and iodide and its application to analysis of rat thyroid hydrolysates.

Methods and materials. Solutions of thyroxine (T_4) 3:3′:5 triiodothyronine (T_3), 3:5 diiodotyrosine (DIT), 3-monoiodotyrosine (MIT), 3:5 diiodothyronine, sodium iodide, tetraiodothyroacetic acid (TETRAC) and triiodothyroacetic acid (TRIAC) were prepared by dissolving each compound in methanol conc. ammonium hydroxide (99:1) so that 1 microliter of each solution contained the equivalent of 1 microgram of iodide. Preparation of the solutions of the individual 131-I labeled compounds (T_4, T_3, DIT, MIT and NaI) was accomplished by the addition of each to methanol:conc. ammonium hydroxide (99:1).

The absorbent was prepared by suspending 30 g of cellulose powder (Whatman C-41) in 75 cc of distilled water which was then slurried for 5 minutes with a magnetic stirrer.

The Desaga Apparatus was utilized to prepare chromatoplates (20 cm × 20 cm) of 0.35 mm thickness. Prior to chromatography the plates were air-dried over night. Drying the plates in a desiccator or pre-heating offered no advantages. For chromatography of the unlabeled iodinated compounds, a known amount of a mixture of the individual compounds was applied to the chromatoplate as a spot less than 5 mm in diameter at the lower left corner 2 cm from each edge using a microsyringe under a stream of air at room temperature. Lines were drawn 16 cm from the point of application for the first dimension, and 11 cm from the point of application at right angles to the first line for the second dimension in order to demarcate the development distance in each direction.

For quantitative recovery studies and autoradiography a known amount of each labeled compound was applied to a chromatoplate as outlined above. A mixture of unlabeled compounds was also applied to the origin for purposes of identification.

The chromatoplates were developed in a standard Brinkman developing chamber by the ascending technique at room temperature. Of the various combinations of solvents examined those found to yield the best separations were: tert. butanol:3% ammon. hydroxide (3:1 v/v) for the first direction. Development time was 4-5 hours. Secondary butanol : glacial acetic acid : water (4:4:1 v/v) was used for the second direction. Development time was 1 hour. The plates were dried at room temperature.

For chemical detection of the iodinated compounds the chromatoplates were sprayed with either 0.25% ninhydrin in acetone or a modification of the ceric-sulphate arsenious acid methylene blue reagent (CAMB)(7) which was prepared using acetone instead of distilled water for the methylene blue solution.

Detection with ninhydrin was utilized to locate spots for later quantitative radioactivity determinations since the use of the CAMB reagent resulted in a significant loss of radioactivity due to formation and sublimation of elemental iodine.

The radiographs of the chromatoplates were produced by exposure of Type M X-ray film (Eastman Kodak) for 72 hours. These were then superimposed on the chromatoplates in order to detect the labeled areas.

The ninhydrin positive spots containing the labeled material were scraped from the chromatoplate with a razor blade and transferred to separate 10 cc test tubes which were placed in a scintillation crystal well-counter with automatic changer and pulse height analyzer for counting at the iodine peak. All samples, including minor contaminants, were counted at preset counts or preset time to a maximum counting error of 1% except for some of the minor contaminants of the DIT and T_3 solutions which were counted to maximum counting errors of 1.9% and 1.4%, respectively, in one of the experiments.

The total radioactivity of the substances applied to the plate was determined by calculating the mean count rate obtained from the same volume of solution placed in each of 8 test tubes which were counted in the same manner as the labeled materials obtained from the plates. The average counts of these sets

TABLE I

Compound	$Rf_{(1)}$ Avg	$Rf_{(1)}$ Range	$Rf_{(2)}$ Avg	$Rf_{(2)}$ Range
Triac	.90	.81-.98	.93	.87-.96
Diiodothyronine	.90	.83-.98	.78	.71-.95
Triiodothyronine	.83	.70-.95	.80	.73-.87
Tetrac	.72	.69-.79	.94	.89-.96
Thyroxine	.67	.50-.79	.81	.75-.89
Sodium iodide	.68	.60-.78	.14	.08-.25
Diiodotyrosine	.10	.06-.16	.69	.64-.75
Monoiodotyrosine	.28	.22-.33	.64	.56-.73

of tubes were used as the standards for each substance.

Rat thyroid hydrolysates were prepared from female ovariectomized 150-200 g Sprague-Dawley rats according to the method of Tong and Chaikoff(8) after equilibrium with 131-I was attained(9). To evaluate the per cent distribution of labeled compounds shortly after 131-I administration hydrolysates were also prepared after a 4-hour time lapse. The hydrolysates were applied to the chromatoplate and the chromatogram developed. The 131-I labeled areas on the plates were detected by autoradiography and quantitation carried out as outlined previously.

Results. Table I shows the Rf values for each of the iodinated compounds in both systems. The positions of the labeled components revealed by autoradiography coincide exactly with those of the stained compounds. Although Rf's varied somewhat the relative position of each compound remained constant. Fig. 1 shows a chromatograph stained with modified CAMB reagent demonstrating the separation of T_4, T_3, Tetrac, Triac, DIT, MIT and NaI. 3-5 diiodothyronine could not be separated from Tetrac in this system. The CAMB reagent was capable of detecting at least .001 μg of iodide on the chromatoplate. Thin-layer chromatography and staining of individual nonradio-labeled compounds revealed single spots in all instances. Individual radio-labeled iodinated amino acids that were similarly treated revealed the presence of small amounts of other iodinated amino acids and iodide which were quantitated by applying the individual labeled iodinated amino acids and carrier solutions to multiple separate plates then removing the individual areas according to the radioautograph and quantitating the radioactivity present. Table II shows the composition and recovery after chromatography of the radio-labeled T_4, T_3, MIT and DIT solutions received from the manufacturer. Results are expressed in per cent $\pm$ standard deviation. The average recovery of total radioactivity from the plates exceeded 94% in all experiments. In each experiment, the major labeled component comprised from 88 to 95% of the radioactivity recovered from the plate. The recovery of the individual iodinated compounds from a given solution was reproducible with a standard deviation of less than 1% of the total radioactivity. A mixture of the radio-labeled

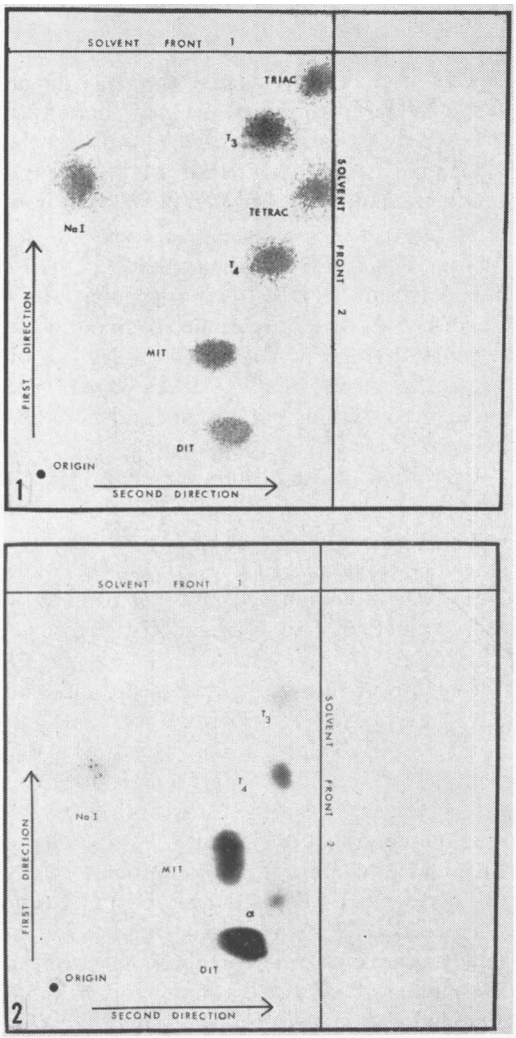

FIG. 1 and 2.

TABLE II. Percent Recoveries (± S.D.) of Components in "Pure" Radio-Labeled Compounds.

Solutions chromatographed		T_4	T_3	DIT	MIT
% Radioactivity of individual components recovered	T_4	87.6 ± .7	1.7 ± .8	<1	<1
	T_3	4.7 ± .3	96.3 ± 1.0	<1	<1
	DIT	<1	<1	95.5 ± .1	1.9 ± .1
	MIT	2.5 ± .3	<1	1.6 ± .3	93.1 ± .4
	I	4.8 ± .2	1.3 ± .3	2.3 ± .3	4.4 ± .4
% of total radioactivity recovered		96.5 ±1.9	98.9 ± 2.2	94.5 ±2.6	100.8 ±1.4
No. of determinations		8	8	7	8

TABLE III. Percent Recoveries (± S.D.) from Mixtures of "Pure" Radio-Labeled Components. Activities and recoveries are adjusted for known composition of components in "pure" radio-labeled compounds.

Compound	T_4	T_3	DIT	MIT	NaI
Activity of each compound added in mixture	12,795	8,962	14,495	13,245	3,217
Activity of each compound recovered from mixture	13,052	8,619	14,718	13,624	3,059
% Recovery of each compound from mixture	102	96.1	101.5	102.9	95.1

iodinated amino acids was chromatographed. Since the compounds as received from the manufacturer were not pure, the amount of each compound applied in the mixture was considered to be the sum of the radioactivities of the specific compounds recovered from individually chromatographed solutions of each of the iodinated amino acids. Table III shows the recovery of the individual compounds from a mixture after chromatography. Activities are expressed in counts per minute. Recovery of all compounds from the mixture exceeded 95%. The composition of solutions of the radio-labeled compounds changed with time in all instances with decline in the per cent of the major component.

Fig. 2 shows an autoradiograph illustrating the clear separation of 131-I labeled components of a rat thyroid hydrolysate. Table IV shows the per cent distribution of 131-I in rat thyroid hydrolysates. The compound alpha was detected in all rat thyroids studied to date. It migrates between MIT and DIT in the first direction and more rapidly than both in the second direction. Average Rf's of alpha are: .22 and .83 in the first and second directions, respectively. The results are in agreement with those obtained from paper chromatography and with published data although our MIT values are lower. This may represent an inability of other systems to separate alpha from MIT. Triac was less than 1% in our system.

In 13 duplicate experiments, recovery of radioactivity from rat thyroid hydrolysate after chromatography averaged 80.2% with a range of 62-109%. Those with duplicate agreement of 10% or less averaged 80% recovery. After areas containing known compounds were removed from the plate, the remainder of the absorbent was scraped off and counted in 3 experiments. Residual radioactivity was 0.5-1% of the total.

Discussion. In this study, standard mixtures of iodinated amino acids and iodide have been clearly separated using TLC. The labeled compounds have been recovered quantitatively.

This technique has been successfully applied to separation and quantitation of labeled amino acids in rat thyroid hydrolysates. Alpha is an unidentified 131-I labeled substance that has been detected in all rat thyroids studied to date. Its nature is unknown but studies have demonstrated that this is not monoiodohistidine. It is not formed on the chromatoplate from alteration of other iodinated compounds since it is found only in the rat thyroid hydrolysates and not in standard mixtures. In the solvents used, the acetic acid derivatives of T_4 and T_3 travel near the thyronines in both directions. It is possible that alpha may be an acetic acid derivative of MIT or DIT since it migrates near the tyrosines.

Faircloth *et al*(3) have studied rat thyroid

TABLE IV. Comparison of Paper and Thin-Layer Chromatographic Methods Showing Distribution of 131-I in Rat Thyroid Homogenates.

	No. of rats	Origin	T_4	T_3	I	DIT	MIT	Alpha
TLC								
Isotopic equilibrium	17	3.9 ± 1.5	14 ± 3.3	2.7 ± 1.2	3.9 ± 1.6	51.8 ± 4.2	20.4 ±30	3.3 ± 1.6
4 hr after 10 μC 131-I	6	3.5 ± 0.4	7.9 ± 1.5	1.6 ± 0.2	5.3 ± 0.2	51.9 ± 2.2	27.2 ± 1.5	2.5 ± 1.9
Paper								
Isotopic equilibrium	15	5.3 ± 2.4	11.1 ± 3.9	1.6 ± 1.4	4.9 ± 1.3	50.7 ± 3.7	26.5 ± 4.4	—

hydrolysates using TLC. Although the separation achieved was excellent, iodide was not recovered quantitatively due to losses from the acidic formic acid : water system. Recovery of compounds averaged 76.0 ± 3.6% of the iodine containing compounds applied to the plates. West *et al*(4,10) measured serum T_4 levels after separation by TLC. The method used did not separate T_3 from T_4 and the published low Rf values seem to indicate that separation and quantitation of all moieties is technically difficult. The method of Shapiro and Gordon(6) gives recoveries similar to ours.

The modification of the CAMB reagent using acetone instead of distilled water prevented distortion of the spots on the chromatoplate. This stain was capable of detecting .001 μg of iodide; however, its use is associated with variable iodine loss, hence is not applicable to quantitative studies.

The advantages of this method are distinct separation of each spot with good resolution and excellent recovery and reproduceability. There was no loss of iodide or alteration of the compounds during chromatography.

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Interferon Production in Hamsters Experimentally Infected with Rabies Virus.* (31568)

WILLIAM E. STEWART II AND S. EDWARD SULKIN
Department of Microbiology, University of Texas Southwestern Medical School, Dallas

The virus inhibitor interferon has been shown to be produced by cells, both *in vivo* and *in vitro,* in response to several viruses and other agents(1). However, no work has

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