THE UPTAKE AND CHEMICAL NATURE OF IODINE COMPOUNDS IN THREE PLANT SPECIES

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IODINE IN PLANTS

KARAGEOZIAN

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AN ABSTRACT OF THE THESIS OF

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Title: The uptake and chemical nature of iodine compounds in three plant species.

Iodine is an essential element in human nutrition; in spite of its universal distribution in nature, goiter occurs in areas where a deficiency of iodine is prevalent. In Lebanon, there is a high incidence of goiter, but little is known concerning the cause or causes of goiter in the Lebanese population. However, recent studies have shown that it was primarily the result of iodine deficiency. The present investigation was initiated to study some possible contributing reasons for iodine deficiency.

In Lebanon, foods of vegetable origin are the major sources of dietary iodine, but little is known concerning the chemical nature of plant iodine or its physiological availability. The present study was conducted to provide more information concerning selected vegetable foods as a source of dietary iodine, by investigating the following parameters:

1. The effect of pH on uptake of radioiodine in three common plant species of Lebanon (mint, Mentha spp.; swiss chard, Beta vulgaris, var. cicla ; chicory, Cichorium intybus).
2. The qualitative chemical nature of the iodine compounds in these green plants.
3. The physiological availability of the iodine in these plants.

The results of the uptake experiments showed that the optimum pH values for maximum iodine uptake in each of the three plants studied was in the acidic range. In all instances uptake was drastically reduced in the neutral and basic media.

Results of paper electrophoresis, paper chromatography and thin layer chromatography showed that all the radioiodine taken up by mint, swiss chard and chicory remained in the inorganic unbound form in the plant tissues.

Thyroidal uptake studies with iodine deficient rats fed radioactive plant material from the three species

showed that the iodine in swiss chard and chicory was readily available; 36-38 percent of the dose fed was taken up by the rat thyroid in 24 hours. The radioactivity in mint was less available; only 20 percent of the dose fed was found in the thyroid after 24 hours.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION AND LITERATURE REVIEW

Courtois, in 1811, first found iodine while preparing nitre from dried seaweed; a few years later, Gay-Lussac named and described the chemical properties of this element (5, p 185). In 1820, Coindet successfully treated patients with goiter (thyroid enlargement) using tincture of iodine, and in 1895 (7), iodine was established as an essential element in human nutrition. However, it was Chatin, who after analysing foodstuffs and water, concluded that, in spite of the universal distribution of iodine in nature, goiter occured in areas where a deficiency of this element was prevalent (5, p 186).

Even though the discovery and use of radioactive iodine has contributed greatly to the elucidation of the various phases of iodine metabolism, the problem of goiter has survived from the day of Chatin to the present. In fact, it is estimated that over 200 million people throughout the world suffer from this condition (10, p 28). The cause is generally associated with a dietary deficiency of iodine; this nutrient is necessary for the synthesis of the hormone, thyroxine, in the thyroid gland. To compensate for this deficiency, the gland becomes more active and enlarges, many times to gross proportions.

Endemic goiter, can be an exceedingly serious

public health problem since often it is associated with cretinism, feeblemindedness, and general physical and mental degeneration (13). In addition, there appears to be a correlation between endemic goiter and a high birth rate of deaf mutes.

In Lebanon, there is a high incidence of goiter. Recent surveys (11 & 9, p 80) have shown that, especially in high mountain valleys, 80 percent of school children may have enlarged thyroids, and even in coastal areas, the condition is not uncommon. Although the problem is serious, little is known concerning the cause of goiter in the Lebanese population. However, two studies have shown indirectly that in the groups studied it was primarily the result of iodine deficiency; in these groups, iodine therapy reduced goiter incidence significantly (4, 12). Moreover, a recent study by the Division of Food Technology and Nutrition at the American University of Beirut, showed that actual 24-hour intake of iodine in three Lebanese villages was far below the minimum daily requirement (3).

The present investigation was initiated, not to study iodine intake or deficiency *per se*, but rather to study some possible contributing reasons for iodine deficiency. In Lebanon, foods of vegetable origin are the major sources of dietary iodine; obviously this iodine must be taken up from the soil by the plant. Analysis of soil samples from all over Lebanon has revealed that, even

though many soils seem high in iodine, green vegetables grown on these soils are low in this element[1]. It is believed that acidic soils yield iodine to plants more readily than do basic soils (1, p 7-9), but no firm proof of this has been published. Thus one of the purposes of this work was to study the effect of pH on iodine uptake in selected plants to see if perhaps soil pH could be related to their ability to concentrate iodine.

Although it is known that green plants under proper conditions readily incorporate iodine into their tissues, little is known concerning the chemical nature of this iodine or its "physiological availability". In other words, iodine in certain plants may be present in a chemical form which is not absorbed and utilized in the body. A preliminary report by Cowan and Esfahani (2) showed that radioactive iodide was taken up readily by lettuce plants and appeared to remain in the leaves in the unbound, inorganic form. The radioiodine thus incorporated was readily absorbed in iodine deficient rats and concentrated in the thyroid glands of the animals. Thus, a further purpose of this investigation was to expand on the work of the latter workers and study the chemical form and physiological availability of iodine in plants other than lettuce.

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1. Unpublished data obtained by Division of Food Technology and Nutrition, American University of Beirut.

In summary, the main objective of the present investigation was to provide more information concerning vegetable foods as a source of dietary iodine by:

1. Studying the effect of pH on iodine uptake in three common plants species of Lebanon (mint, Mentha spp.; swiss chard, Beta vulgaris, var. cicla; chicory common, Cichorium intybus).

2. Establishing the qualitative chemical nature of the iodine compounds in these green plants.

3. Studying the physiological availability of the iodine in these plants.

II. MATERIALS AND METHODS

Incorporation of radioiodine into the plants

Seedlings of the three plant species (mint, swiss chard and chicory) were removed carefully from soil and were transferred to 500 ml brown jars containing tap water. The plants were kept in this medium with frequent changes of the water for 12-14 days or until they developed healthy roots. At that time the water was replaced by Hoglands' nutrient medium (8, p 85). One ml of carrier-free radioactive[1] NaI^{125}, containing 125 microcuries (uc) of radioactivity was added per 500 ml of the nutrient medium. Initially, the desired pH of each individual jar was established by addition of the appropriate amount of either 0.01N sulfuric acid or 0.01N sodium hydroxide; the pH was checked daily and adjusted accordingly. The plants were grown in these media for 3 weeks at which time the upper stems and leaves were removed. A portion of the radioactive plant material was dried for determination of the uptake by the plants and for physiological availability studies in rats; the remainder of the fresh tissue was extracted as described below for chromatographic studies.

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1. Obtained from the Radiochemical Center, Amersham, England.

Drying samples for radioactivity determinations

The radioactive plant material was dried in a gravity convection oven at 50°C for 48 hours after which the individual dried samples were thoroughly ground in a mortar. Precisely 0.10 g of each ground sample was weighed into a clean test tube and the radioactivity measured in counts per minute (cpm) using a well-type, crystal scintillation counter[1]. The counting system had a background of 140 ± 10cpm and a counting efficiency of about 40 percent.

Water extraction of leaf samples

Ten g of the fresh leaves of the radioactive plants were triturated thoroughly for 5 minutes in a mortar with 10 g of purified sea sand. After trituration, 50 ml of distilled water were added and the whole mass was triturated further. The mixture was centrifuged at 3000 revolutions per minute (rpm) for 10 minutes and the supernatant liquid retained. This procedure was repeated three times using a fresh 50 ml portion of distilled water each time. The pooled supernatant liquid thus collected after each extraction was filtered under suction through Whatman No. 1 filter paper. The filtrate was frozen at -18°C and lyophilized. After lyophilization the freeze-dried powder was dissolved in 0.5 ml distilled water; this

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1. Baird-Atomic Inc., Model 135, Cambridge, Mass., U.S.A.

concentrate was used for chromatographic studies.

Ammonium hydroxide extraction of leaf material

The leaf material was triturated initially with sand as described above after which the entire mass was extracted with 4 successive 30 ml portions of diethyl ether. The ether was discarded and the extracted material was triturated further with 50 ml of 1.5N ammonium hydroxide solution. As in the water extraction procedure, the mixture was centrifuged at 3000 rpm and the supernatant liquid retained. This extraction procedure was repeated three times, each time using fresh 50 ml portions of the ammonium hydroxide solution. The supernatant liquid collected from each extraction was pooled, filtered under suction and lyophilized. The dry powder was dissolved in 0.5 ml of 1.5N ammonium hydroxide and the resultant concentrate used for chromatographic studies.

Ether and chloroform extraction of leaf samples

One g of the dried leaves of the radioactive plants grown at the optimum pH's was continuously extracted in a Soxhlet for 16 hours, using ether and chloroform as the extraction solvents. The extracts were concentrated by evaporating the excess solvent; the remaining concentrate was retained for radioactivity measurements.

Electrophoresis

For the electrophoresis experiments, the instrument

used was an L.B.K., model 3276-50[1]. Sheets of Whatman No. 1 filter paper, 180x420 mm, served as chromatograms; the papers were run for exactly 2.5 hours at 300 volts and 3.7 milliamperes in veronal buffer of pH 8.6 (2.76 g of diethyl barbituric acid + 15.4 g of sodium diethyl barbiturate per liter of buffer solution). Before the current was applied, the filter paper was soaked in the buffer and the soaked paper was allowed to equilibrate in the apparatus for 1 hour. After equilibration, the concentrates of the plant extracts, as well as a known sample of sodium iodide, were applied and the current turned on. When completed, the chromatograms were dried at room temperature and developed with either the FFCA reagent (see page 9) or by spraying with a 0.1 percent solution of ninhydrin in chloroform.

Paper chromatography

Strips of filter paper (type L.B.K. 3276, Carl Schleicher and Schull No. 2024) 40x300 mm were used for the chromatograms. Two developing solvents were employed: 1) a mixture of n-butanol, glacial acetic acid and water (12:3:5 v/v); and 2) a mixture of collidine and water (100:36 v/v). An aliquot of the concentrates prepared by either water or ammonium hydroxide extraction of leaf samples was applied under a stream of cool air to the

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1. L.B.K. 3276 Produkter AB. Box 76, Stockholm - Bromma 1, Sweden.

origin of the chromatogram; the chromatograms were run one dimensionally with the solvent ascending. For reference, a known sample of radioactive NaI^{125} was chromatographed separately and cochromatographed with the leaf extracts. The chromatograms were run for 10 hours after which they were dried at room temperature and developed either by autoradiography or by using a developing mixture of ferric chloride ($FeCl_3.6H_2O$), potassium ferricyanide ($K_3Fe(CN)_6$), and sodium arsenite ($NaAsO_2$) (6); hereafter, this latter mixture will be referred to as the "FFCA reagent".

FFCA reagent

The FFCA reagent was prepared by mixing the proper proportions of ferric chloride, potassium ferricyanide and sodium arsenite (6). The dry chromatograms were dipped into the mixture followed by thorough washing with tap water; they were then dried at room temperature. To prevent discoloration of the chromatograms, the entire developing procedure was conducted in a dark room. The developed spots, which indicated organic and inorganic iodine or certain other reducing agents, acquired a blue color on a white or very light blue background.

Thin layer chromatography

Glass plates (200x200 mm) coated with a 0.25 mm layer of silica gel G were used for the chromatograms. After the wet gel was applied, the plates were activated

in a gravity convection oven at 110°C for 12 hours; the plates were then cooled and stored in a desiccator. The developing solvents used were the same two described under "Paper chromatography" above. An aliquot of the concentrates prepared by either water or ammonium hydroxide extraction of leaf samples was applied under a stream of cool air to the origin of the chromatograms; the chromatograms were run one dimensionally with the solvent ascending. For reference, a known sample of radioactive NaI^{125} was chromatographed separately and cochromatographed with the leaf extracts. The chromatograms were run for 5 hours, after which they were dried at room temperature and autoradiographs were made from them. After the autoradiographs were prepared, the chromatograms were sprayed with the 0.1 percent ninhydrin reagent.

Autoradiography

Radioautographs were prepared by placing Kodak medical X-ray film (blue brand 25x43 cm) on the chromatograms (either paper or glass plates) and storing in lightproof cardboard exposure folders for 5 days. After exposure, the films were developed in a dark room with Kodak supermix X-ray developer and fixed with Kodak supermix X-ray fixer.

Assessment of physiological availability of plant iodine

The experimental animals used were adult albino

rats of the Sprague-Dawley strain[1]. The animals were rendered iodine deficient by maintaining them for 6-8 weeks on a low iodine diet (Table 1) which was provided ad. libidum. During this period, the animals were housed in meshbottom cages, in an air-conditioned room held at 21 ± 1°C and relative humidity of about 60 percent.

The physiological availability of the radioiodine incorporated into the plants was assessed by measuring iodine uptake by the thyroid gland of iodine deficient rats which were fed measured amounts of the radioactive material. Prior to the uptake experiments, the animals were starved for 36 hours. Then, a measured amount of each ground radioactive sample was administered either by gelatin capsule or by allowing the animal to eat a small amount of the basal low iodine diet to which the radioactive material had been added. After administration of the radioactive food, the animals were kept for 24 hours in individual metabolic cages for collection of urine and feces. The animals were then sacrificed; radioactivity was measured in the thyroid, urine, feces, and intestinal contents.

The thyroid of each sacrificed animal was digested in 1 ml of alcoholic-potassium hydroxide (5 g KOH in 50 ml of 50 percent ethanol) for 48 hours at 38°C. After digestion, radioactivity in each thyroid was measured in the

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1. Obtained from Animal Suppliers, (London), Ltd.

Table 1. Composition of basal low iodine diet

Ingredients	Amount
	%
Corn starch	60
Casein	20
Corn oil	10
Non-nutritive cellulose (Alpha cel)[1]	5
Mineral mixture (Iodine-free)[2]	4
Vitamin mixture[1]	1

1. Obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A.

2. U.S.P. XIV, less potassium iodide; obtained from General Biochemicals Inc., Chagrin Falls, Ohio, U.S.A.

well-counter. For the determination of radioactivity in the urine samples, aliquots were counted directly. The feces and the intestinal contents of the sacrificed animals were pooled and homogenized in a Virtis "45" blender-homogenizer; an aliquot of the homogenate was taken for radioactivity measurement.

III. RESULTS AND DISCUSSION

Selection of plants

The three plants (mint, swiss chard and chicory) used for experimentation were selected for the following reasons:

a. The three plants represent a large category of green leafy vegetables.

b. They are known to be relatively high in iodine[1].

c. They are used commonly as food in Lebanon.

Experiment I.

The effect of pH on iodine uptake in plants

Seedlings of the three plants were grown in media of various pH levels as described under Materials and Methods. At the end of three weeks, radioactivity was determined in each seedling; the data are summarized in Table 2. It is clear from Table 2 that the optimum pH for maximum uptake of radioactive NaI^{125} by mint was about 4.5; as the pH was increased to 8.0, the uptake value decreased by a factor of ten relative to that at 4.5. In the case of swiss chard, the optimum pH for maximum uptake was

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1. Unpublished data obtained in the Division of Food Technology and Nutrition, A.U.B.

Table 2. The effect of acidic and basic media on the uptake of iodine by three plant species.

pH of Nutrient Media[1]	Radioactivity[2]		
	Mint	Swiss chard	Chicory
	cpm	cpm	cpm
4.0 ± 0.1	85,300	*	20,700
4.5 ± 0.1	108,700	92,400	10,500
5.0 ± 0.1	91,700	129,400	7,500
6.0 ± 0.1	75,300	163,700	7,100
7.0 ± 0.1	23,600	61,100	3,800
8.0 ± 0.1	10,400	28,300	2,600

1. Each flask contained 125 uc radioactivity.

2. Measured in 1.0 g dry material, each value represents the average of triplicate determinations.

*. Plants died at pH 4.0.

about 6.0, while for chicory the optimum value was about 4.0. The results indicate that, for each of the three plant species considered, the optimum pH for maximum iodine uptake was different. However, all the optimum values were in the acidic range, and in all instances, uptake was lower in neutral and basic media. The reasons for this observation are not clear and further work is needed to study the movement of iodides into root tissues under conditions of varying pH's.

It is not clear why each plant should have a different optimum pH for maximum uptake. Furthermore, the fact that relative uptake of iodine in mint and swiss chard was more than in chicory cannot be explained. Perhaps species differences would account for these latter observations; considering literature values available, chicory does appear to be lower in iodine than swiss chard (1, p 75 and 88) and mint[1].

Experiment II.

Chemical identification of plant iodine

It was assumed that the radioiodine taken up by the plants was in the water soluble fraction of the plant material. However, to investigate the possibility that some was present in the lipid fraction, two dried samples

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1. Unpublished data, F.T.N. laboratories, A.U.B.

each of the three plants were extracted with ether and chloroform. No radioactivity was recovered in either extract.

Method of extraction

Experiments with water extraction techniques indicated that 70-75 percent of the total radioactivity could be extracted from the plant material. Since about 25 percent of the radioiodine could not be extracted by water alone, it was evident that some other and better solvent had to be used. A solution (1.5N) of ammonium hydroxide was chosen; by using this procedure it was possible to extract 92-95 percent of the radioiodine from the plant material. No proof was obtained to show that there was no breakdown of possible organic iodides under these conditions.

The chemical form of the radioiodine in the plants was investigated in the extracts by using paper electrophoresis and chromatography and thin layer chromatography. Autoradiographs were prepared for the detection of radioactive compounds on the chromatograms. In addition, the FFCA reagent was used to detect, qualitatively, inorganic iodides and iodinated organic compounds. Since iodinated amino acids are found in animal tissue, ninhydrin was also used to see if amino acid spots corresponded with radioactivity on the chromatograms.

The FFCA reagent is very sensitive to micro amounts of iodine, but it has the drawback that it responds also to other reducing agents such as phenols. However, by using

the three detection methods (autoradiography, FFCA and ninhydrin), it was possible to obtain conclusive results.

Paper electrophoresis

Water extracts of the three plants were electrophoresed and the papers were developed with the FFCA reagent. When the plant extracts were run for 6 hours, no FFCA spots were detected on the paper; however, by decreasing the time to 2.5 hours, one major FFCA-sensitive spot developed, with each of the three plant extracts, 17-18 cm from the origin (Table 3). The distance traveled by these spots was similar to that of the standard sodium iodide (Table 3), indicating that the iodine in the plants was in the inorganic form.

Further evidence that the iodine was inorganic in nature was the fact that, with all the plants, the FFCA sensitive spot moved far out in front of any ninhydrin-sensitive area.

Although the evidence obtained with electrophoresis indicated that at least most of the plant iodine was inorganic, the results were not completely satisfactory for two reasons: 1) there was always a certain amount of "tailing" of the extract on the paper, and 2) the standard sodium iodide always moved somewhat ahead of the plant extracts. Therefore, since there was still the possibility of finding organically bound iodine in the plants, paper chromatography were used in an attempt to derive more

Table 3. Paper electrophoresis of plant extracts and standard sodium iodide.

Materials	FFCA Peaks Distance from origin[1].
	cm
Mint	17.30[2]
Swiss chard	17.40[2]
Chicory	17.10[2]
Sodium iodide	18.00[2]

1. Origin on electrophorogram was 9.0 cm away from one edge of paper.

2. Did not correspond with any ninhydrin-sensitive area.

conclusive evidence.

Paper chromatography

The results of paper chromatography using n-butanol/ acetic acid/ water as solvent are presented photographically in Figures 1-6. Figures 1, 3 and 5 represent the results obtained by using the FFCA reagent with mint, swiss chard and chicory, respectively. In Figure 1 there are four strips of paper: A, water extract of mint; B, standard sodium iodide; C, ammonium hydroxide extract; and D, a cochromatogram of ammonium hydroxide extract and standard sodium iodide. There are a number of intense blue or light blue bands shown by letters (a), (b) or (c); these bands indicate the presence of iodine compounds (or other reducing compounds) in the plant tissues. Comparing bands on the chromatograms of water and ammonium hydroxide extracts with the band produced by the standard sodium iodide, it is clear that the four bands marked (b) in the four strips have the same Rf value and that these bands correspond to sodium iodide.

As for bands (a) and (c), at this stage it was not possible to determine whether or not these were iodine compounds. To resolve this question, autoradiographs of the strips were prepared; the one for mint is shown in Figure 2. The autoradiographs have only one band which corresponds to that of band (b) (sodium iodide) in Figure 1; bands (a) and (c) were non-radioactive but had the property

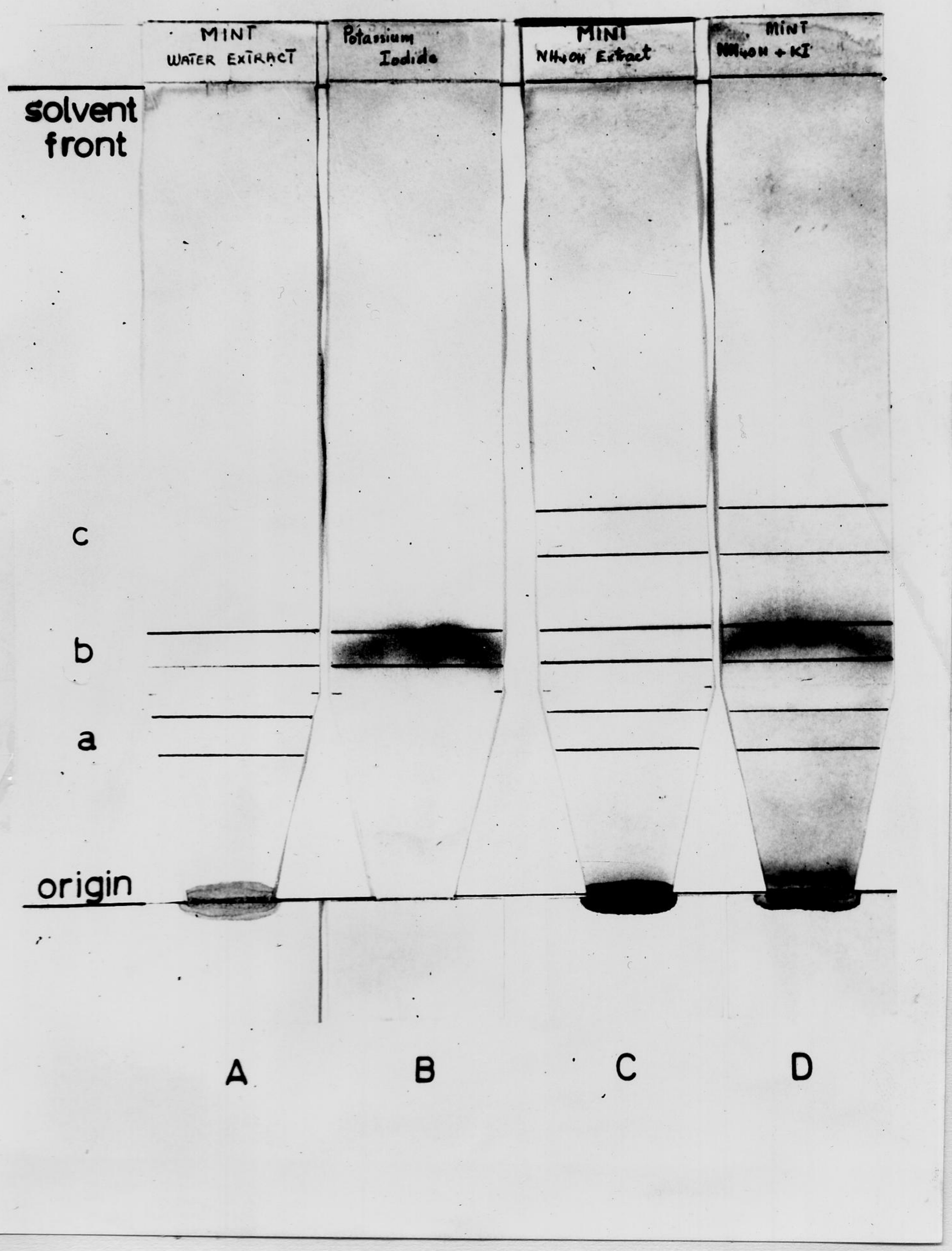

Figure 1. FFCA peaks on paper chromatograms of mint plant extracts chromatographed in n-butanol/acetic acid/water solvent system.

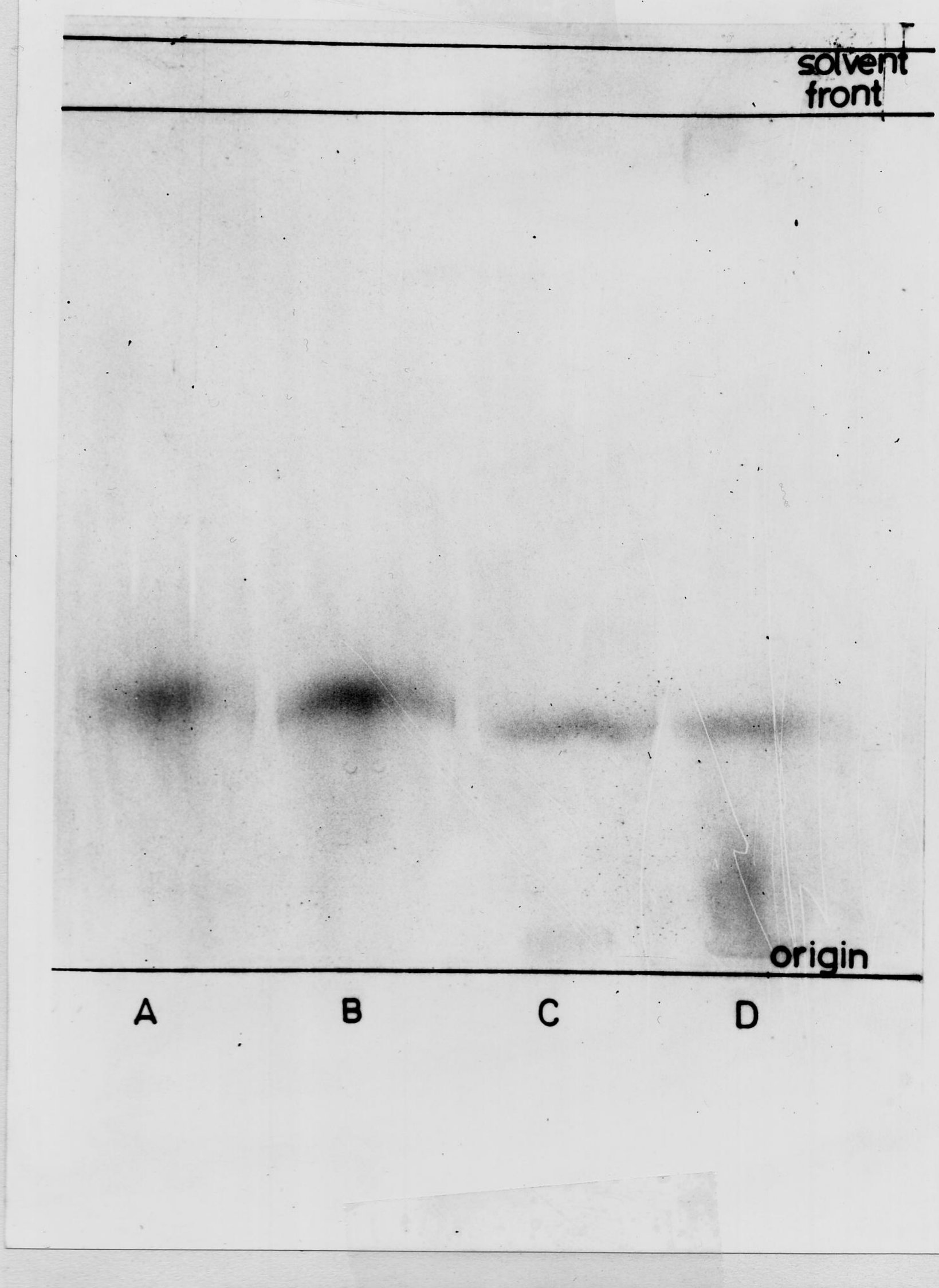

Figure 2. Autoradiograph of paper chromatograms of mint plant extracts chromatographed in n-butanol/acetic acid/water solvent system. A = water extract; B = standard NaI^{125}; C = ammonium hydroxide extract; D = ammonium hydroxide extract + NaI^{125}.

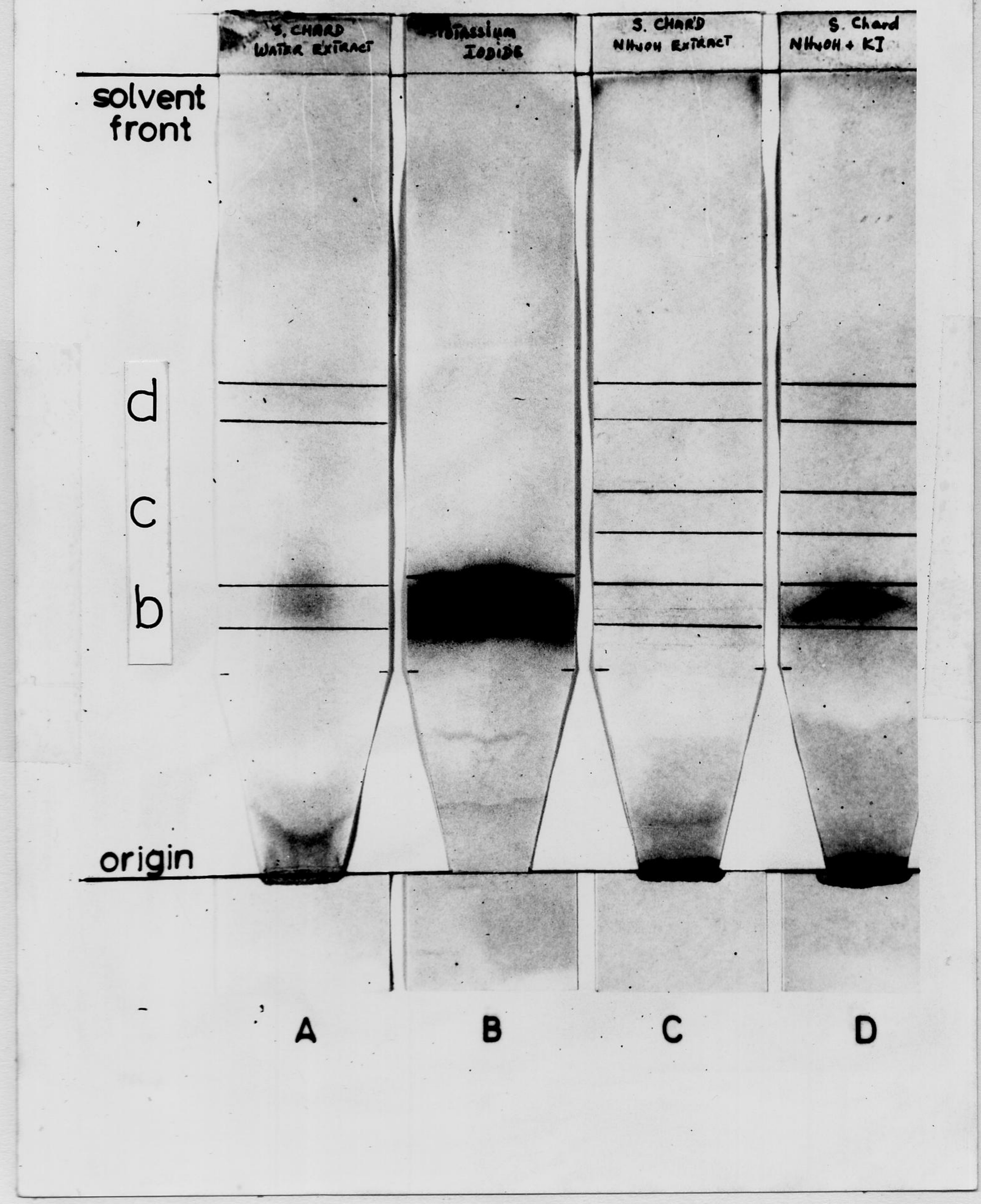

Figure 3. FFCA peaks on paper chromatograms of swiss chard plant extracts chromatographed in n-butanol/acetic acid/water solvent system.

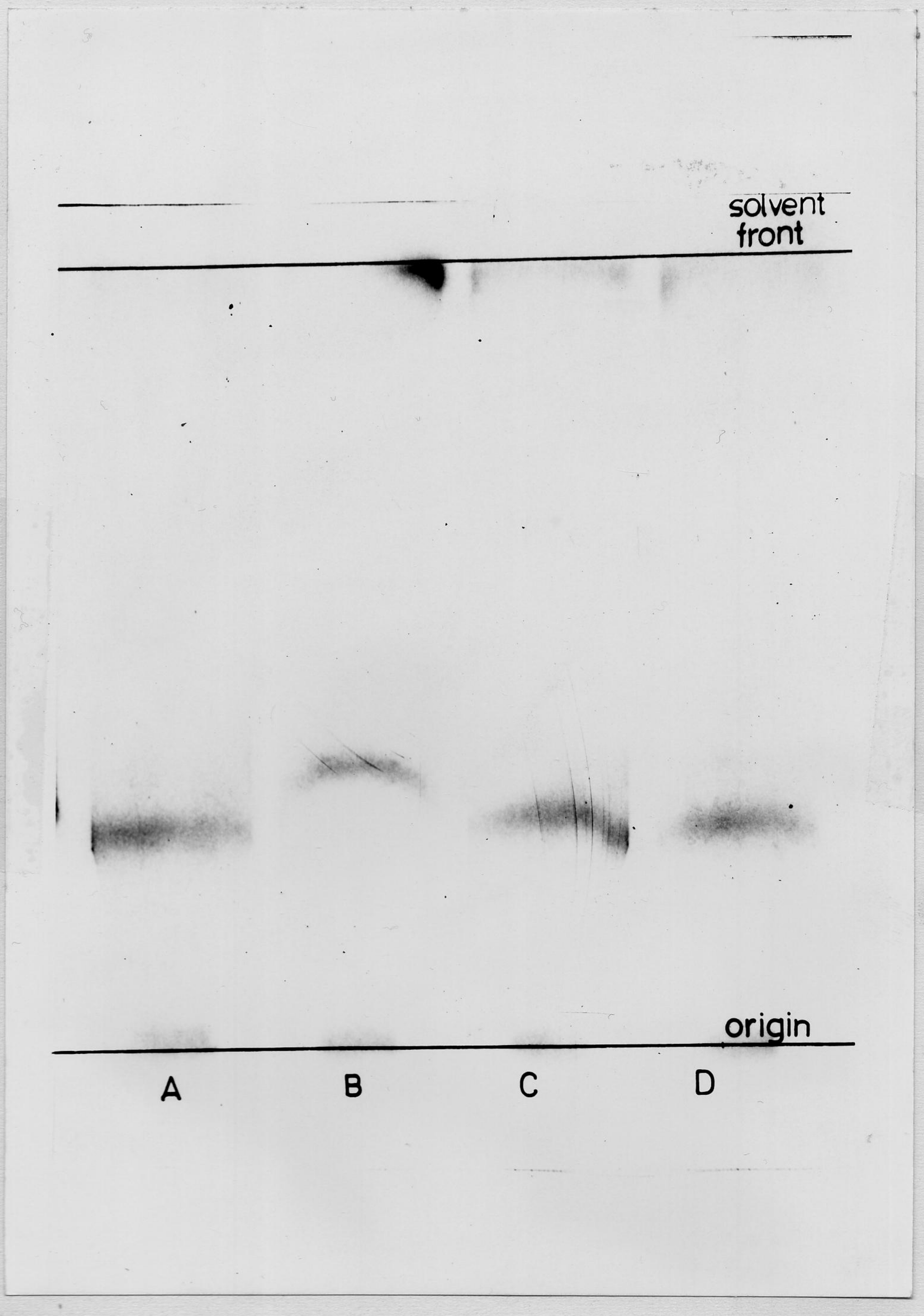

Figure 4. Autoradiograph of paper chromatograms of swiss chard plant extracts chromatographed in n-butanol/acetic acid/water solvent system. A = water extract; B = standard NaI^{125}; C = ammonium hydroxide extract; D = ammonium hydroxide extract + NaI^{125}.

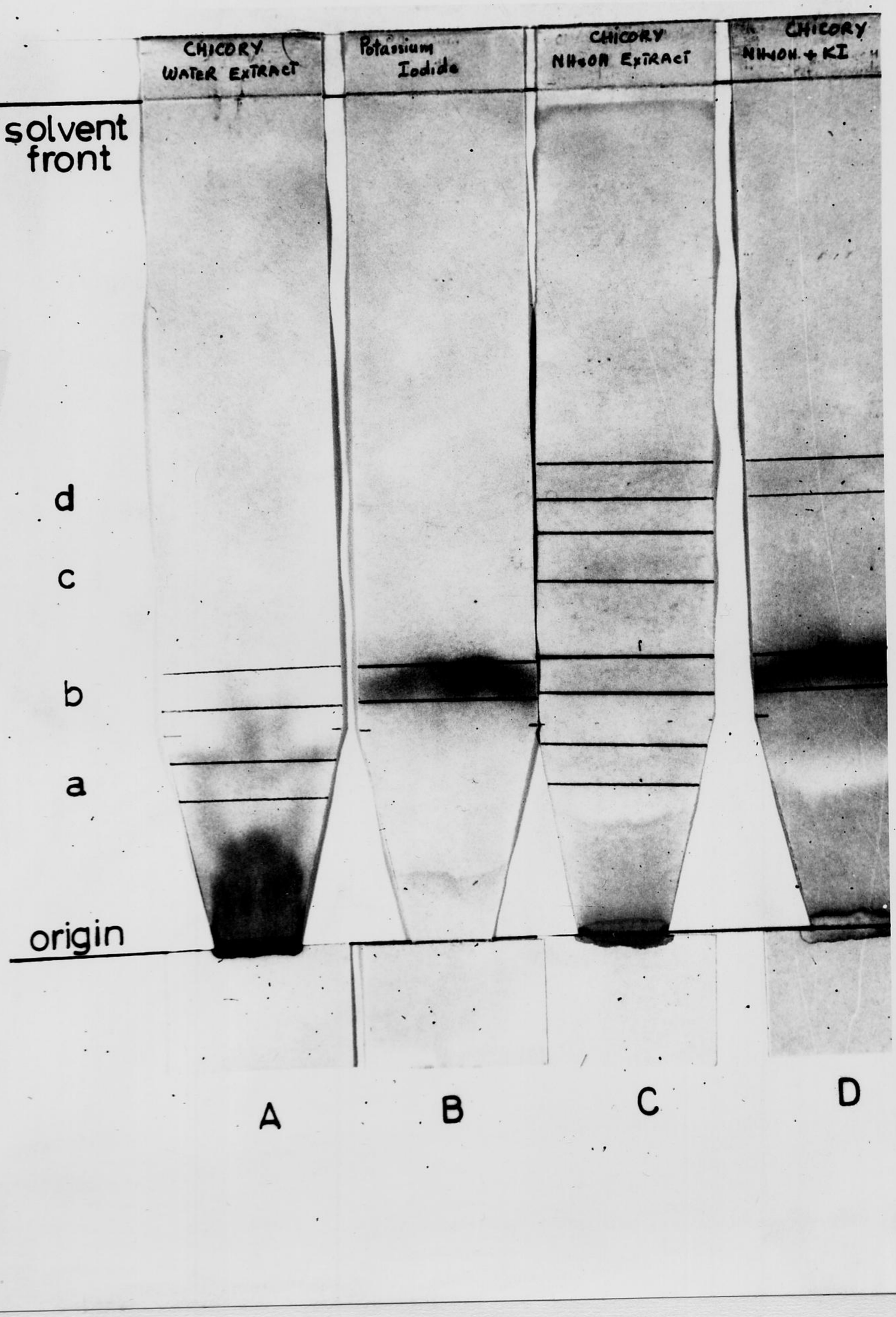

Figure 5. FFCA peaks on paper chromatograms of chicory plant extracts chromatographed in n-butanol/acetic acid/water solvent system.

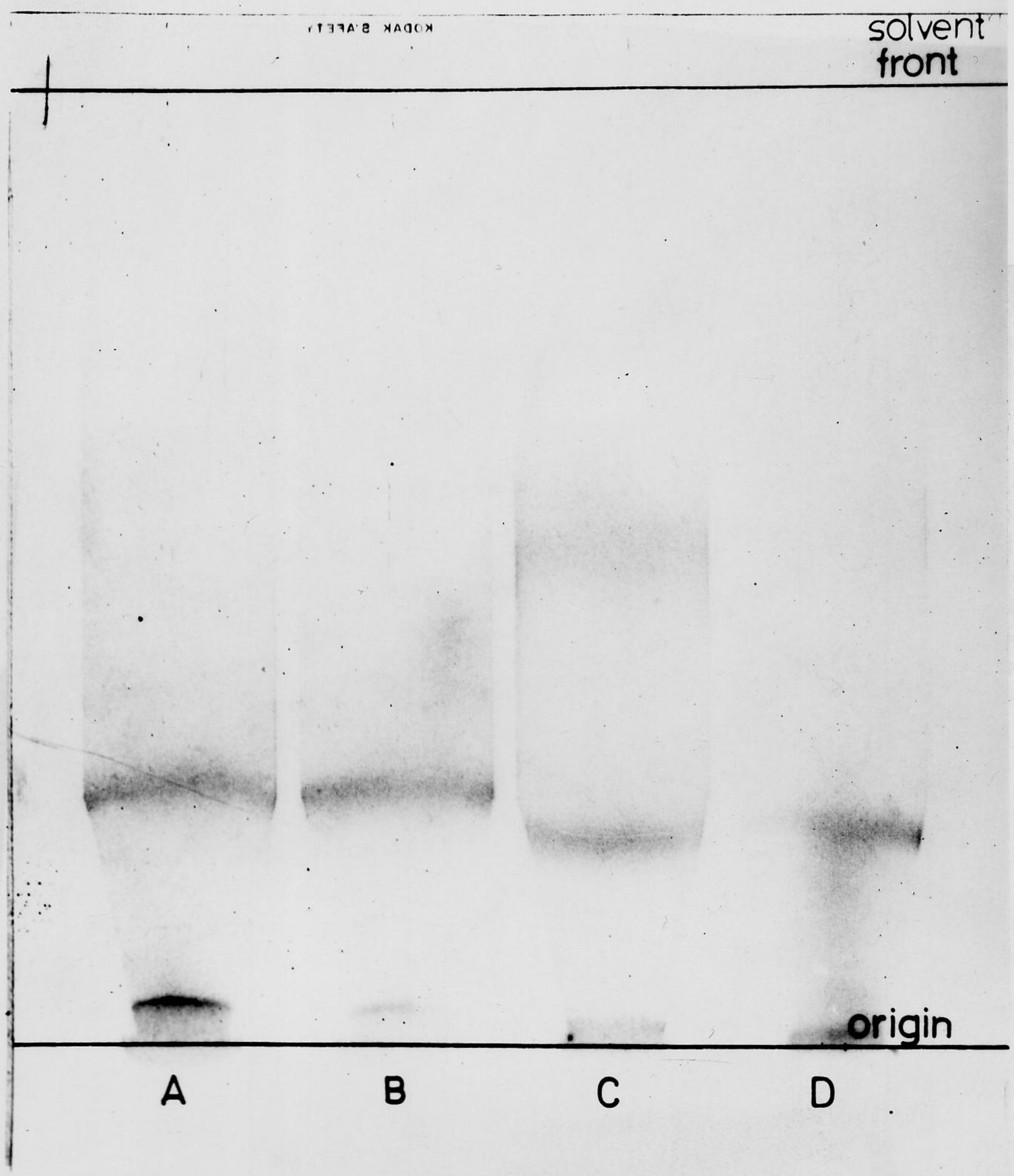

Figure 6. Autoradiograph of paper chromatograms of chicory plant extracts chromatographed in n-butanol/acetic acid/water solvent system. A = water extract; B = standard NaI^{125}; C = ammonium hydroxide extract; D = ammonium hydroxide + NaI^{125}.

of reducing the FFCA reagent. If bands (a) and (c) had been iodine compounds, they would have marked the film; since they did not, they can be considered as non-iodine compounds.

The results obtained with swiss chard (Figures 3 and 4) and with chicory (Figures 5 and 6) were the same as with mint; all the radioiodine incorporated into the plants remained as inorganic iodide in the plant tissues. It should be noted that, on the FFCA chromatograms of the chicory extracts (Figure 5), there was, in addition to bands (a), (b) and (c) a fourth band (d) which was FFCA-sensitive but non-radioactive.

On all the paper chromatograms developed in n-butanol/ acetic acid/ water and sprayed with ninhydrin, it was noted that the peak of radioactivity coincided with a ninhydrin-sensitive area. Thus, even though evidence in favor of inorganic iodine was strong, it was not clear cut. Therefore, a second solvent system (collidine/water) was tried to see if the radioactive spot would move away from any ninhydrin-sensitive areas. The results summarized in Table 4 show the values for the FFCA peaks using the collidine/water solvent system. These data show that the peaks coincide with standard sodium iodide but not with any ninhydrin-sensitive area. Thus, it appeared that in all three plants none of the iodine was organically bound, and that the results obtained with butanol/acetic acid/water

Table 4. Rf values of FFCA peaks on paper chromatograms of mint, swiss chard and chicory plant extracts chromatographed in a collidine-water solvent system[1].

Material	Rf values		
	Mint	Swiss chard	Chicory
Water extract	0.636	0.641	0.636
Ammonium hydroxide extract	0.639	0.641	0.640
Ammonium hydroxide extract + NaI	0.639	0.646	0.640
Sodium iodide	0.636	0.639	0.636

1. None of the spots corresponded with ninhydrin-sensitive areas.

were coincidental.

The Rf values of the radioactive spots obtained by autoradiography of the paper chromatograms developed with collidine/water are summarized in Table 5. One radioactive peak appeared per paper and, in all instances, the Rf values of the plant extracts coincided with the Rf of standard NaI^{125}. Also, when NaI^{125} was chromatographed with the ammonium hydroxide extracts, the known compound moved identically with the radioactivity in the extracts (Table 5).

The results obtained with paper chromatography supported those of the electrophoresis work and indicated that the radioiodine incorporated into the seedlings of the three plants studied remained as inorganic iodide in the plant tissues. Although this evidence appeared conclusive in itself, there were discrepancies encountered such as: 1) the coincidence of the radioactive peaks with ninhydrin-sensitive areas when butanol/acetic acid/water was used; and 2) the slight differences in Rf values obtained (see Table 4). Thus, it was decided to subject the extracts to another method of resolution (thin layer chromatography) to see if these difficulties could be overcome.

Thin layer chromatography

The thin layer chromatograms were run in the same two solvents mentioned previously; the radioactive spots

Table 5. Rf values of radioactive spots on paper chromatograms of mint, swiss chard and chicory plant extracts chromatographed in collidine-water solvent system[1].

Material	Rf values		
	Mint	Swiss chard	Chicory
Water extract	0.636	0.641	0.639
Ammonium hydroxide extract	0.640	0.641	0.642
Ammonium hydroxide extract + NaI^{125}	0.640	0.643	0.642
Sodium $iodide^{125}$	0.636	0.641	0.639

1. None of the radioactive spots coincided with ninhydrin-sensitive areas.

were detected by preparing autoradiographs of the chromatograms. Also, the chromatograms were sprayed with ninhydrin to detect the positions of the ninhydrin-sensitive spots. Because it was impossible to dip the plates, development with FFCA reagent was not attempted.

Autoradiographs of the chromatograms of mint, swiss chard and chicory run in butanol/acetic acid are shown in Figures 7, 8 and 9, respectively. Also, results obtained using collidine/water are shown in Table 6. It is clear from Figures 7, 8 and 9 and from Table 6 that, in all instances, the radioactive spots of the plant extracts moved identically with the standard NaI^{125} in both solvents. Furthermore, the radioactive areas are far removed from any ninhydrin-sensitive spots.

These results support those obtained with both electrophoresis and paper chromatography. It was concluded that, under the experimental conditions used, radioiodine incorporated into mint, swiss chard and chicory remained in the inorganic, unbound form.

Experiment III.

Physiological availability

The physiological availability of the radioiodine taken up by the plants was assessed in adult albino rats. To insure maximum utilization of this nutrient, animals were rendered iodine deficient prior to receiving the

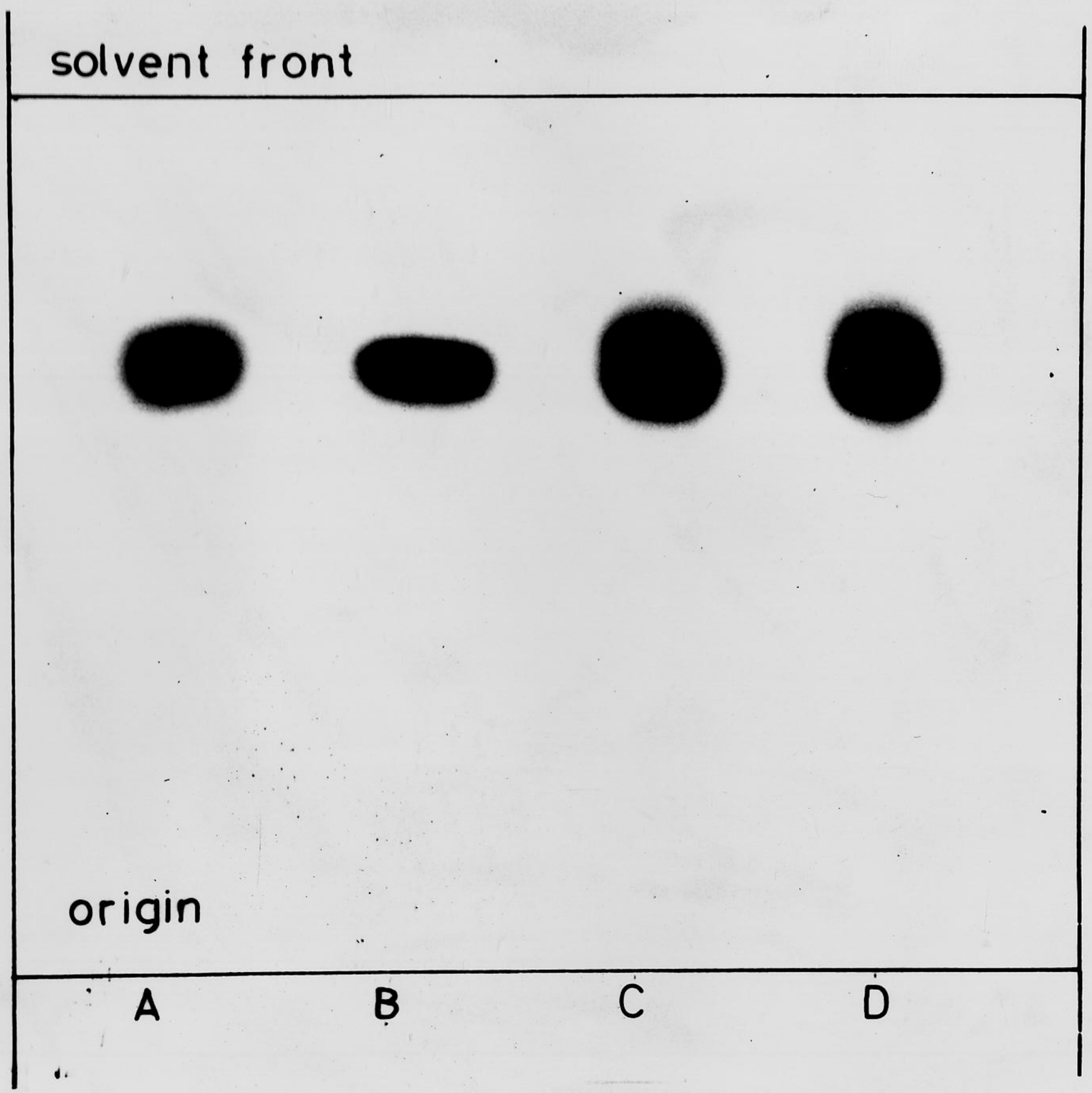

Figure 7. Autoradiograph of thin layer chromatogram of mint plant extracts chromatographed in n-butanol/acetic acid/water solvent system. A = water extract; B = standard NaI^{125}; C = sodium hydroxide extract; D = ammonium hydroxide extract + NaI^{125}.

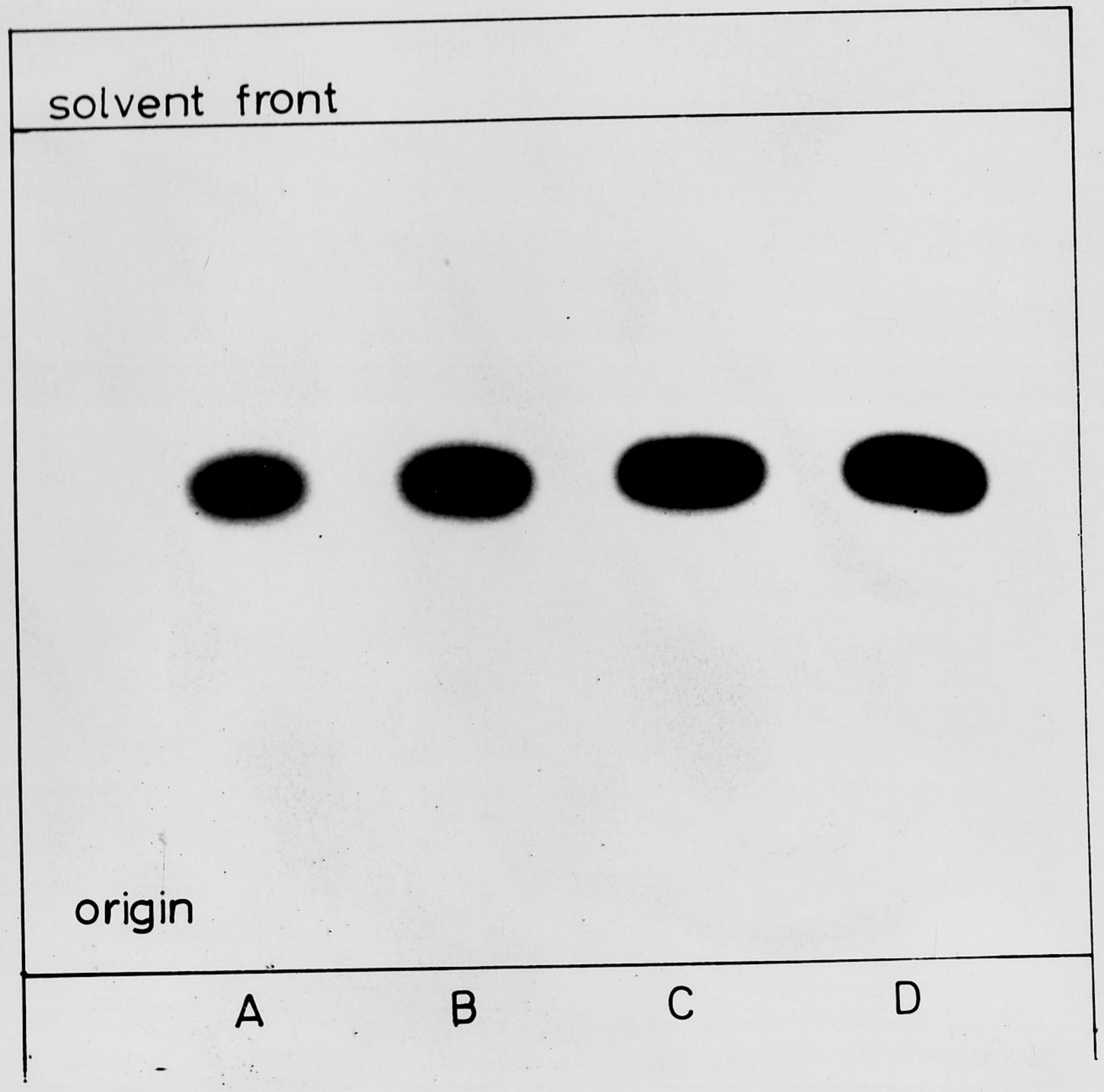

Figure 8. Autoradiograph of a thin layer chromatogram of swiss chard plant extracts chromatographed in n-butanol/acetic acid/water solvent system. A = water extract; B = standard NaI^{125} ; C = ammonium hydroxide extract; D = ammonium hydroxide extract + NaI^{125}.

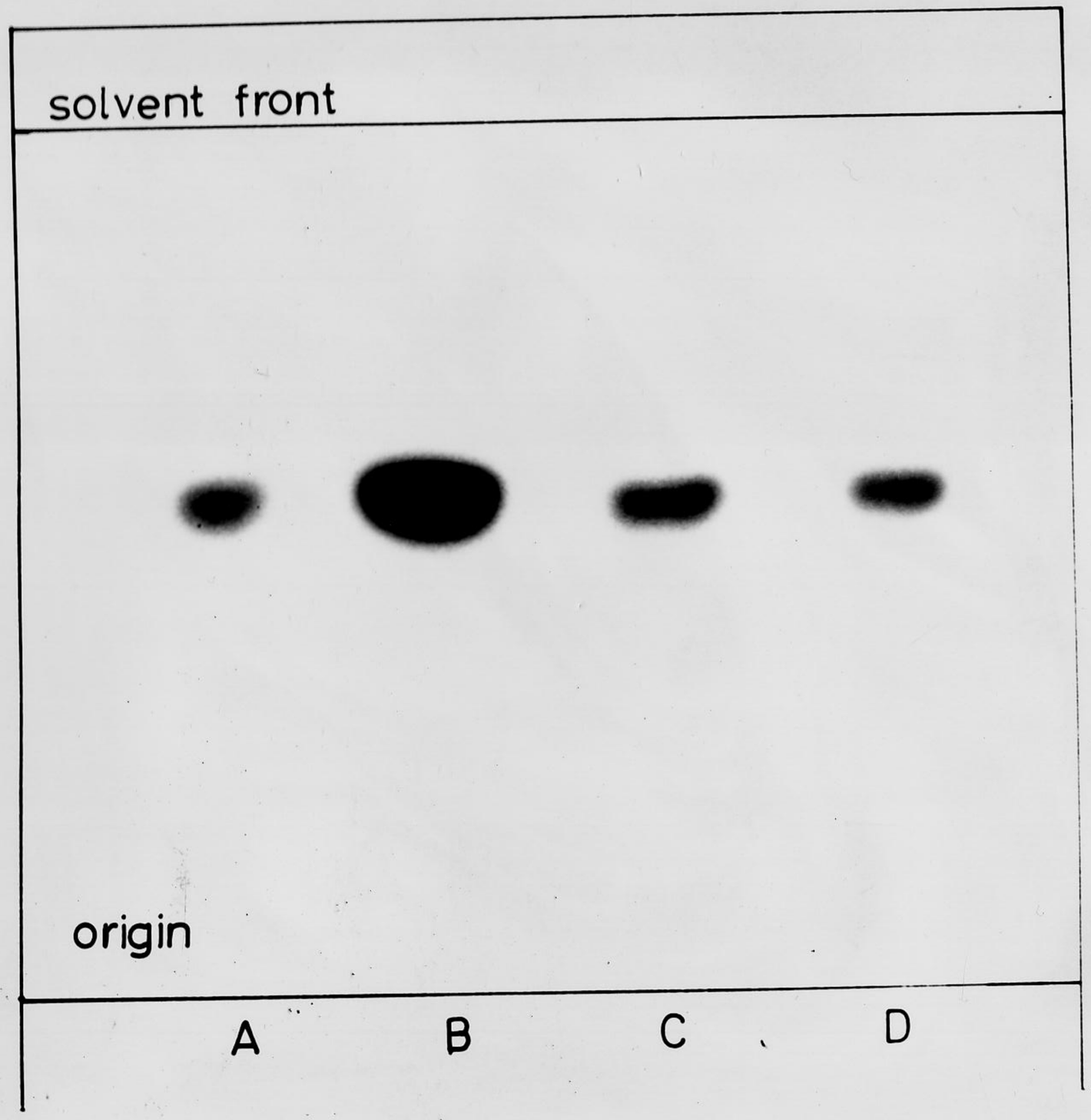

Figure 9. Autoradiograph of a thin layer chromatogram of chicory plant extracts chromatographed in n-butanol/acetic acid/water solvent system. A = water extract; B = standard NaI^{125}; C = ammonium hydroxide extract; D = ammonium hydroxide extract + NaI^{125}.

Table 6. Rf values of radioactive peaks on thin layer chromatograms of mint, swiss chard and chicory plant extracts chromatographed in a collidine-water solvent system[1].

Material	Rf values		
	Mint	Swiss chard	Chicory
Water extract	0.875	0.876	0.875
Ammonium hydroxide extract	0.873	0.874	0.873
Ammonium hydroxide extract + NaI^{125}	0.873	0.875	0.873
Sodium $iodide^{125}$	0.879	0.875	0.877

1. None of the radioactive spots coincided with ninhydrin-sensitive areas.

radioactive plant material. The results of the animal experiments are summarized in Table 7. With swiss chard and chicory, 35-38 percent of the original radioiodine fed was taken up by the thyroid, and a portion of it was excreted in the urine and feces. With mint, the availability was less; only 20 percent of the original dose given was taken up by the thyroid. However, a slightly greater amount of radioactivity was excreted in the feces. In the case of radioactive NaI^{125}, which was used as a control, 45-47 percent was taken up by the thyroid.

From the results of this experiment, it appears that the iodine in swiss chard and chicory was readily available since the uptake compared favorably with that of NaI^{125}. The data on mint showed that the iodine availability was relatively low, perhaps because the plant material was somewhat less digestible.

Although the radioiodine in the plants seems to be readily absorbed and concentrated in the thyroids of the rats, further studies are needed to see if this iodine is "physiological" in nature, that is, if it is actually incorporated into circulating thyroid hormone.

General discussion

The results of this limited study indicate that soil pH may well be a contributing factor to the iodine deficiency problem in Lebanon. If soil iodine behaves like the iodine in the nutrient media used, green plants

Table 7. The uptake and excretion of radioiodine in mint, swiss chard and chicory by iodine deficient rats.

Material	Radioactivity							
	Mint		Swiss chard		Chicory		Sodium iodide ^{125}	
	1	2	3	4	5	6	7	8
Dose fed (cpm)	1730	1400	2960	3230	2240	1940	6500	6500
Thyroid (percent)	20	19	35	37	36	38	47	45
Urine (percent)	9	10	8	8	6	7	12	13
Feces[1] (percent)	32	29	26	27	29	26	2	3

1. Included intestinal contents.

grown on soils of high pH (7 and above) would not be expected to take up much of this element. It is known that many of the soils in Lebanon are calcarious (high pH); therefore plants normally considered rich sources of iodine would not be such when grown on these soils.

Further studies are needed to assess the physiological availability of plant iodine in humans. However, if iodine actually gets into the plant tissues, and if the results of the present physiological availability studies in rats can be interpreted in terms of the human, green plants may be considered good sources of available iodine. Thus, it may be speculated that, in Lebanon, by proper soil treatment, plants that are now poor sources of iodine could have their iodine levels doubled or trebled. However, the economical aspects of such treatment would be hardly feasible; a far better approach to improving the status of iodine nutrition in Lebanon would seem to be the iodization of table salt.

IV. SUMMARY AND CONCLUSIONS

The purpose of this study was to provide more information concerning green leafy vegetables as a source of dietary iodine. Three different experiments were performed to investigate:

1. The effect of pH on uptake of radioiodine in three common plant species of Lebanon (mint, swiss chard and chicory).

2. The qualitative chemical nature of the iodine compounds in these green plants.

3. The physiological availability of the iodine in these plants.

The results of experiment I showed that the optimum pH values for maximum iodine uptake in the three plants were in the acidic range. In all instances, uptake was drastically reduced in neutral and basic media.

In experiment II the results obtained from paper electrophoresis, paper chromatography, and thin layer chromatography showed that all the radioiodine taken up by mint, swiss chard, and chicory remained in the inorganic, unbound form.

When radioactive swiss chard and chicory were fed to iodine deficient rats (Experiment III), 36-38 percent of the radioiodine was taken up by the rat thyroids in

24 hours. The radioiodine in mint was somewhat less available; 20 percent of the dose given was in the thyroid after 24 hours.

The results of this study are discussed in relation to the problem of iodine deficiency in Lebanon.

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