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METHODS FOR STUDY OF IMIDAZOLE COMPOUNDS AND APPLICATION TO BRAIN AND CANCER CELLS*

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Abstract—Two-dimensional thin-layer chromatographic (TLC) methods were developed for the separation of seventeen imidazole compounds of biological interest. Depending on the combinations of these compounds present in the mixture to be analyzed, separations could be achieved employing four different types of TLC plates and eleven solvent mixtures. Visualization procedures were described for the detection of imidazoles both with unsubstituted and substituted rings, and suitable ancillary radioautographic and counting procedures were described for use with labeled substances. A quantitative procedure employing the diazo reaction with sulfanilic acid was developed for the determination of imidazole compounds with an unsubstituted ring after elution from cellulose and Silica gel TLC plates. The above techniques were applied to the identification of imidazoles in extracts of brain of several species as well as to the study of the metabolism of histidine and *N*-acetyl histidine in Ehrlich ascites cells.

ALTHOUGH there has been great recent interest in various imidazole compounds found in the nervous system, with the exception of histidine there is little definitive information about any specific intracellular function performed by any one of them. One of the most severe constraints under which workers in this field have been working has been the lack of rapid methods for the separation, sensitive detection and quantitative determination of various imidazole compounds. Because of our current interest in the metabolism of histidine, histamine and related substances in brain[1–5] and histidine in cancer cells,[6] we have begun to address ourselves to the above problems. The present paper deals with the development of separation and detection methods for several imidazole compounds of biological interest and in some instances for their quantitative determination.

MATERIALS AND METHODS

Equipment and thin-layer chromatographic (TLC) plates. The following items of equipment were found to be useful: Desaga migration chamber for electrophoresis of thin-layer plates and multiplate rectangular tanks (Brinkmann Instruments, Inc., Calif.); Drummond disposable micropipettes, 1, 2 and 5 μl (Van Waters & Rogers, Los Angeles); and Shandon type spray gun (Brinkmann Instruments, Inc.). The following TLC plates were found to be useful for our work: Silica gel F254 and 254, Avicel (on aluminum), Cellulose (on glass) (E. Merck AG, all obtained from Brinkmann Instruments, Inc.) and Ecteola (on plastic) (J. T. Baker Chemical Co.).

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Chemicals. All of the imidazole derivatives were obtained either from CalBiochem (La Jolla, Calif.) or Sigma Chemical Co. (St. Louis, Mo.). Fast Red B and 4-aminobenzoic acid were purchased from Sigma; ninhydrin was purchased from Pierce Chemical Co. (Rockford, Ill.); *o*-phenylenediamine HCl and sulfanilic acid from Eastman–Kodak (Sargent Welch, Anaheim, Calif.); and *N*-bromosuccinimide from Matheson Coleman & Bell (East Rutherford, N.J.). All other reagents and solvents were purchased from J. T. Baker Chemical Co. (Van Waters & Rogers).

All standards were prepared in twice distilled water or in 10% isopropanol (in twice distilled water). Usually standards were made up to contain 10 μmoles/ml of imidazole derivative.

Tissue samples were removed quickly, weighed and immediately homogenized in five volumes (w/v) of 6% perchloric acid. After centrifugation to remove the precipitate, the supernatants were heated in a 90° water bath for 10 min, cooled and brought to a pH of approximately 5 with solid K_2CO_3. The precipitated $KC10_4$ was removed by centrifugation, the samples were frozen overnight, carefully thawed, and the additional precipitate was removed by centrifugation. Then the samples were evaporated on a rotary evaporator at or below room temperature, and taken up in a small amount of water (0·5–1·0 ml). It was found useful to dilute brain samples so that a 1-μl sample contained extract equivalent to 1000 μg of fresh weight of brain.

Thin-layer chromatographic procedures. Deproteinized biological samples and standards are applied with 1-μl pipettes on a corner spot 2 cm from two of the sides of a TLC plate. The spot can be dried after application either *in vacuo* or in a stream of dry air or dry nitrogen, but never with heat. As many applications can be made as are necessary to achieve appropriate sample size. The chromatography generally should be performed at some constant temperature between 20 and 26°. After each run the TLC plate is removed and dried, first in the hood to remove most of the solvent, and then in a vacuum dessicator containing Silica gel, paraffin and either KOH pellets or solid boric acid for acid or alkaline solvents respectively.

Electrochromatography. In a number of instances the best separations were achieved with cellulose plates on glass, when electrophoresis was employed in the second dimension. The optimal electrolyte for this purpose in our hands consisted of a solution of 1% acetic acid and 1% formic acid, final pH 2·0. Another useful solution was 0·1 M ammonium formate, pH 3·2. These could be used most successfully if chromatography in the first dimension had been carried out in an acid solvent. The dry TLC plates are sprayed very lightly with the electrolyte solution and the moist plate is placed on the electrophoresis apparatus so that the edge with the sample is on the anodic side, since under the above conditions the substances of interest undergo a cathodic migration. Electrophoresis is continued for at least 30 min at 500–800 V. After the electrophoresis, the TLC plate is dried rapidly in a drying oven until almost dry. Final drying is performed in a vacuum desiccator.

Radioautographic and counting procedures. We have found that TLC plates prepared in the above manner are suitable for radioautography. However, special precautions must be taken with plates which have been exposed to formic acid, since the latter interferes with film development. Longer periods for drying are recommended in the latter case. The dried chromatograms are placed in a cassette with an 8 × 10 inch sheet of Kodak Pan Film No. 4155 (Estar thick base) for 3 weeks and the film is developed at 25° in a Kodak D-11 developer for 8 min and, after rinsing in a stop

bath (1 min), fixed in Kodak general fixer for 3 min. The imidazole compounds are located by spraying with the appropriate reagent and their positions compared with the radioactive areas. Approximately 3000 dis/min of $^{14}C/cm^2$ was found to produce an easily visible spot upon 3 weeks of exposure. The radioactive spots can be scraped off the plates, collected in a Swinnex filter fitted with a microweb filter (Millipore Corp., Bedford, Mass. 01730), and counted by liquid scintillation techniques to determine the amount of radioactivity in the sample. The scrapings are placed in counting vials with 1 ml of methanol–1·0 M Hyamine (2:1) solution. After about 15 min, 15 ml of fluor solution is added. The liquid scintillation fluor contains 3 g of 2,5-diphenyl-oxazole (PPO) and 100 mg of 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)] benzene (POPOP) per liter of toluene.

Visualization reagents. Based on the reaction between imidazole and bromine,[7] we have developed a procedure that can detect imidazoles both with unsubstituted and substituted rings. The completely dry TLC plate, dried at 100°, is sprayed lightly with a freshly prepared aqueous 1% solution of *N*-bromosuccinimide to each 100 ml of which had been added 1 ml of bromine-saturated water. Immediately after spraying, the TLC plate is heated in an oven at 100° for 3–4 min, removed, and resprayed lightly with the *N*-bromosuccinimide solution, and then sprayed immediately with a freshly prepared 1% aqueous solution of *o*-phenylenediamine dihydrochloride. Imidazole compounds are visualized in visible light as white spots on red–brown, sometimes greenish background. Under short u.v. the spots appear as white to light blue on a red–brown to black background. The limits of sensitivity of the method varied from one imidazole compound to another, ranging from 5 to 20 nmoles/0·5 cm^2 spot. In tests with fifteen amino acids and serotonin, negative results were obtained with all but threonine and serotonin; but, unlike the imidazoles, the latter two substances gave spots darker than the background. To our knowledge the above procedure is the first one that can detect both unsubstituted and substituted imidazoles on TLC.

4-Aminobenzoic acid-$NaNO_2$ reagent[8] is employed for detection of imidazole compounds in which there is an unsubstituted ring. The dry TLC plate is sprayed with a 1:1 mixture of ice-cold 1% 4-aminobenzoic acid in 2 N HCl and a freshly prepared ice-cold aqueous solution of 5% $NaNO_2$. The mixture must stand 10–15 min on ice before use. The plates are allowed to dry at room temperature and then are sprayed with a 20% solution of Na_2CO_3 to bring out the typical red color. All diazotizable imidazole compounds tested gave red colors with this reagent with the exception of urocanic acid and imidazoleacetic acid, which varied from red to brown. Histidine, carnosine and imidazole acetic acid were detected at levels of 0·2 nmole, while the limit of detection for other imidazoles was approximately 0·4 nmole/cm^2 spot.

Sulfanilic acid (*p*-aminobenzenesulfonic acid) also is employed for the detection and quantitative estimation of imidazole compounds in which there is an unsubstituted ring. The dry TLC plate is sprayed with a 1:1 mixture of ice-cold 1% sulphanilic acid in 10% HCl and a freshly prepared ice-cold aqueous solution of 5% $NaNO_2$. The plates are allowed to dry at room temperature and then sprayed with a 20% Na_2CO_3 solution in water to bring out the red color.

Fast Red B (5-nitro-2-aminoethoxybenzene diazotate) is a particularly good reagent for detecting unsubstituted imidazoles because it is sensitive and tends to give a characteristic color with each substance. The reagent is prepared immediately before use by weighing out 0·6 g into a spray bottle, wetting the salt with 0·5 ml of 96%

ethanol, and then dissolving in 100 ml of distilled water. The TLC plate first is sprayed lightly with 20% Na_2CO_3 solution and is dried at room temperature for 30–60 min. It then is sprayed with the reagent until the colored spots appear. Most imidazoles were detectable at 0·5 nmoles/cm^2 spot; but somewhat larger amounts of histamine, histidine, carnosine, homocarnosine and histidine methyl ester were required.

Ninhydrin is useful for the detection of non-diazotizable histidine derivatives with an amino group such as L-1-methylhistidine, 1-methylhistamine, anserine, etc. To 50 ml of a freshly prepared 0·2% solution of ninhydrin in absolute alcohol are added 10 ml of glacial acetic acid and 2 ml of collidine (2,4,6-trimethylpyridine). Immediately before use, 3 ml of a 1% cupric nitrate solution in absolute alcohol is added to the above. The TLC plate is sprayed lightly with the reagent and the plate left to develop at room temperature, or the appearance of the spots can be accelerated by heating in an oven. A lighter background usually is obtained when the development is at room temperature.

Ring-substituted imidazole compounds without amino groups, such as methylimidazole acetic acid, can also be visualized by dipping the plates in a solution of 0·1% iodine in CCl_4. The color fades rapidly and, therefore, spots located in this manner are best outlined by pencil for subsequent reference.

Urocanic acid (imidazole-4-acrylic acid) has a high u.v. absorption at 240–260 nm and migrates well in the majority of solvent systems developed by us. Therefore, the location of the latter substance under u.v. light can give an excellent indication of how well a particular run has gone and can give the approximate location of a substance on the plate whose R_f values relative to urocanic acid are known. In this manner it is possible under certain conditions to remove a given substance from the plate without spraying with a chromogenic agent, if this should be desirable. In the latter instance, marker urocanic acid can be added to the sample if this substance is not present originally. This procedure is particularly useful for the identification and elution of substances for counting subsequent to radioautography.

Quantitative procedure employing the diazo reaction with sulfanilic acid in the test tube and on TLC plates. The procedure developed is suitable for the quantitative determination of imidazole compounds with an unsubstituted ring in pure solution or after elution from cellulose F and Silica gel TLC plates in aliquots of samples containing 5–40 nmoles. In the past the conventional diazo reaction has not been suitable for this kind of application because of instability of the color. We have overcome this difficulty by employing caffeine as stabilizer, as suggested for the quantitative determination of bilirubin.[9]

The chromatograms for quantitative study are developed as described in the preceding section employing aliquots of known solutions containing 5–40 nmoles of diazo-reactive imidazole derivative or aliquots of unknown materials just below the amounts in which interference with chromatography is noted. Since most biological samples usually have been found to contain only a small number of detectable substances, it often is feasible to employ one-dimensional runs to achieve the desired separations. In the latter instance, several determinations can be made on one TLC plate.

After the plates are completely dry, they are sprayed lightly with the sulfanilic acid reagent. While still damp, the TLC plates are sprayed lightly with a 2:1 mixture of 20% Na_2CO_3 and 5% caffeine. The latter solution is prepared by dissolving 50 g of

caffeine, 75 g of sodium benzoate, and 125 g of sodium acetate in 800 ml of distilled water at 50–60°, cooling, and bringing the volume to 1 liter with distilled water. The reactive imidazole derivatives appear as yellow to orange spots. Too heavy spraying with the latter solution results in the formation of red spots and interference occurs with the subsequent quantitative determination. The plates are allowed to dry up to the point at which they show a damp appearance. The individual spots then are carefully scraped loose with a blunt stainless steel spatula and aspirated into a Swinnex filter holder fitted with a microweb filter disc, and the material is transferred to a centrifuge tube. To the material in the tube is added 0·1 ml of 1 N HCl followed by 0·2 ml of the ice-cold 1 : 1 sulfanilic–nitrite mixture. After 10 min, 0·5 ml of the caffeine mixture is added followed by 2 ml of the Na_2CO_3 solution and the contents are mixed with the aid of a Vortex mixer. Immediately after centrifugation the color in the supernatant is read at 510 nm against a suitable blank prepared from a non-chromogenic region of the same plate. Standards must be run through the whole procedure in the same manner as the unknown.

RESULTS AND DISCUSSION

Conditions for separation of several known imidazole compounds. In Table 1 is shown the summary of the conditions developed for the separation and identification of several imidazole compounds of biological interest. These results were obtained empirically from tests with many hundreds of combinations of solvents and types of TLC plates. The recommended solvent combinations should be used in the given

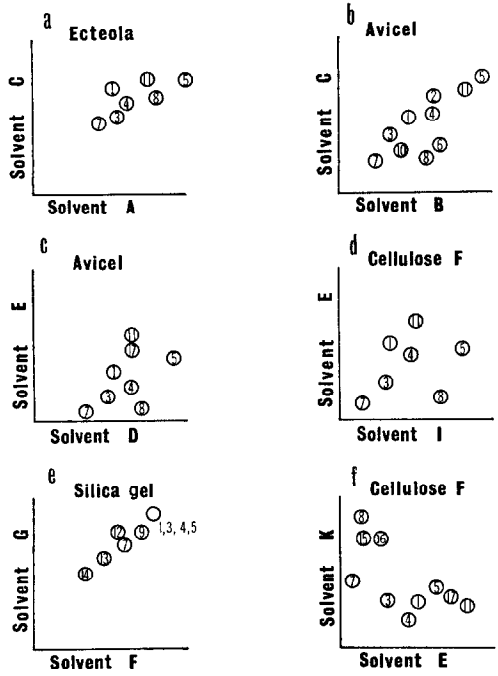

FIG. 1. Tracings of typical chromatograms obtained with the methods described in the text and in Table 1. The numbers in the circles refer to compounds identified by the same numbers in Table 1 and the solvent designations also are given in the above table.

TABLE 1. CONDITIONS FOR SEPARATION OF VARIOUS IMIDAZOLE COMPOUNDS BY TLC

Compounds separated*	Type of plate	Solvents†		Spray reagent‡	Example in Fig. 1
		First dimension	Second dimension		
1, 3, 4, 5, 7, 8, 11	Ecteola	A (5)	C (4)	I	a
1, 2, 3, 4, 5, 6, 7, 8, 10, 11	Avicel	B (6)	C (4)	I§	b
7, 8, 10	Avicel	B (6)	C (4)	II	c
1, 2, 3, 4, 5, 7, 8, 11	Avicel	D‖ (9)	E (5)	I or III	c
1, 3, 4, 5, 7, 8, 11	Cellulose	C (5)	E (5)	I or III	d
		I (6)	E (5)		
1, 3, 4, 5, 7, 8, 11, 15, 16, 17	Cellulose	E (5)	J (0·5)	I or III	f
		E (5)	K (0·5)		
1, 3, 4, 5, 7, 8, 11	Silica gel	A (7)	C (5)	I or III¶	
7, 9, 12, 13, 14	Silica gel	F (7)	H (5)	II	e
		F (7)	G (5)		

* 1, Imidazoleacetic acid; 2, 1-methylimidazoleacetic acid; 3, imidazole lactic acid; 4, *N*-acetylhistidine; 5, *N*-acetylhistamine; 6, 1-methylhistamine; 7, histidine; 8, histamine; 9, L-1-methyl histidine; 10, L-3-methylhistidine; 11, imidazole-4-acrylic acid (urocanic acid); 12, anserine (β-alanyl-L-1-methylhistidine); 13, carnosine (β-alanyl-L-histidine); 14, homocarnosine (γ-aminobutyryl-L-histidine); 15, histidinol; 16, histidine-*O*-methyl ester; 17, imidazolepropionic acid (dihydrourocanic acid). All except 2, 9, 10 and 12 can be visualized with Fast Red B or 4-aminobenzoic-$NaNO_2$ reagent.

† A, *n*-propanol–glacial acetic–water (70:1:30); B, isopropanol–lithium chloride, 0·1 M (70:30); C, *n*-butanol–ethyl acetate–glacial acetic–water (1:1:1:1); D, isopropanol–ammonium acetate, 1·0 M, pH 6·3 (70:30); E, *p* dioxane–*n*-butanol–water–formic acid (40:40:15:5); F, ethanol (100%)–ammonia (28%)–water (70:5:25); G, chloroform–methanol–ammonia (28%)–water (30:60:10:10); H, chloroform–methanol–ammonia (28%)–formaldehyde (37%)–water (30:60:1·5:5:13·5); I, methanol–ethyl phenylacetate–ammonium acetate, 1·0 M, pH 6·3 (90:30:30); J, ammonium formate, 0·1 M, pH 3·2; and K, formic acid (1%) and acetic acid (1%), pH 2·0. Solvents J and K only were employed for electrophoresis. The numbers in parentheses refer to the hours of running time.

‡ I, 4-aminobenzoic-$NaNO_2$ reagent; II, ninhydrin reagent; III, Fast Red B reagent. The preparation of all reagents is described in the text under Materials and Methods. In most instances reagents I and II could detect 0·2 to 5 nmoles of reactive imidazole.

§ Methylimidazole acetic acid was visualized by dipping the plates in a 0·1% iodine in CCl_4 solution or by spraying with *N*-bromosuccinimide followed by treatment with *o*-phenylenediamine as described in the text under Materials and Methods.

‖ Solvent D should be used at 25° or higher.

¶ In this instance Fast Red B gives somewhat better results than the 4-aminobenzoic-$NaNO_2$ reagent.

sequences. The same results are not necessarily observed when the sequences are reversed. Examples of tracings of typical chromatograms with known compounds employing different solvent systems are shown in Fig. 1. The samples were spotted at the point of intersection of the drawn boundary lines. The first dimension of the run was from left to right and the second from bottom to top. The numerical designations given to the various substances in Fig. 1 correspond to those in Table 1.

Quantitative methods. The present procedure shows advantages over the usual test tube procedures for performing the diazo reaction when aliquots of solutions containing imidazole derivatives are employed instead of scrapings from TLC plates. In Fig. 2 are shown the time-courses of color development for 50 nmoles of *N*-acetylhistidine when using the usual conditions for the diazo reaction or when employing the caffeine stabilization. It is seen that the color develops rapidly and fades rapidly under the usual conditions, but that in the presence of caffeine it reaches a stable

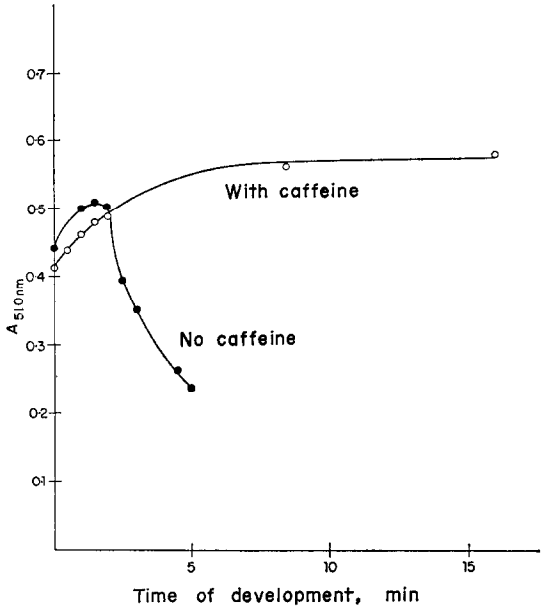

FIG. 2. Time course of color development for 50 μmoles of *N*-acetylhistidine employing the diazo reaction with sulfanilic acid in the presence and absence of caffeine, as described in the text.

maximal reading between 5 and 10 min. Under the above conditions, linearity was achieved between color formation and quantity of material present for each of several substances tested in solution: imidazoleacetic and imidazolepropionic acids, histidine and histamine and their *N*-acetyl derivatives, carnosine, homocarnosine and histidinol. However, a standard curve must be run for each compound determined since the molar color yield varied from substance to substance. Where different amounts of *N*-acetylhistamine were chromatographed one-dimensionally on cellulose F plates employing solvent F (see Table 1) and when *N*-acetylhistidine and imidazoleacetic acid were determined from two-dimensional chromatograms run on four separate cellulose plates on glass employing solvents D and E, linearity of color yield with amount of substance was achieved. In a variety of experiments we found that the range 10–50 nmoles per spot gave us the most accurate results. The influence of actual tissue extracts on the quantitative determination of *N*-acetylhistidine, a typical imidazole, was tested. In Fig. 3 are shown the results obtained from triplicate determinations on three different cellulose plates in which spots of amounts of extract equivalent to 5, 10 or 15 mg of fresh weight of frog (*Rana pipiens*) brain were placed and to which were added 0, 10, 20, 30 or 40 nmoles of *N*-acetylhistidine. The plates were run one-dimensionally in solvent C (see Table 1). The values for absorbancy obtained in the absence of added *N*-acetylhistidine were proportional to the amount of extract used, while the color yields for the added *N*-acetylhistidine were identical in all instances and proportional to the amounts of added *N*-acetylhistidine. Similar results were obtained when various quantities of *N*-acetylhistidine or homocarnosine were added to an aliquot (5 μl) of trout brain extract equivalent to 5 mg of fresh brain and the results compared with those obtained in the absence of added brain extract. In the latter instance, the chromatograms were run one-dimensionally on Silica gel

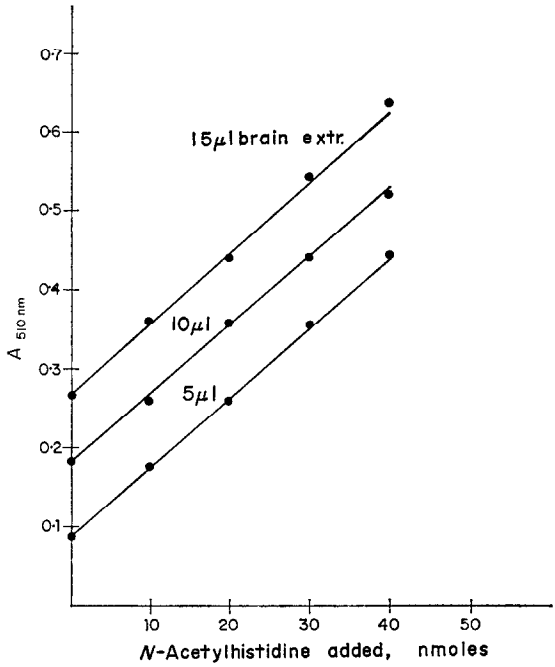

FIG. 3. Amounts of frog brain extract up to 15 mg of fresh wt equivalent do not interfere with color development with *N*-acetylhistidine run one-dimensionally on cellulose plates with solvent C.

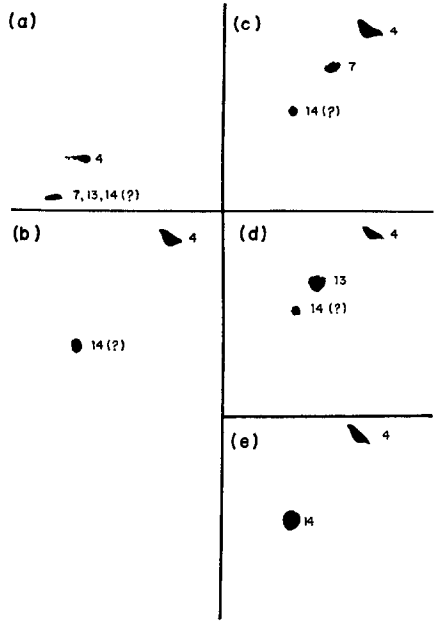

FIG. 4. Steps in the TLC identification of homocarnosine and *N*-acetylhistidine in extract of brain of Rainbow trout. Details described in text. (a) chromatography on Avicel with solvents D and E; (b) chromatography on Silica gel with solvents F and G; (c) same as (b) with the exception that a marker of histidine was added; (d) same as (b) with the addition of carnosine as a marker; (e) same as (b) with homocarnosine added.

plates with solvent F (see Table 1). The color yield was proportional to the amount of imidazole compound and the amount of tissue extract employed did not interfere with color formation.

Application of some of the above procedures to brain extracts. Presumptive identification of homocarnosine and *N*-acetylhistidine was made in extract of brain of Rainbow trout (*Salmo gairdnerii*). The steps taken to achieve the identification are shown in Fig. 4. An aliquot of a perchloric extract equivalent to 16 mg of original fresh weight of brain was chromatographed two-dimensionally on Avicel using solvents D and E (see Table 1), respectively, and visualized with the diazo reaction (Fig. 4a). Comparison with the positions of known substances under the above conditions suggested that the upper spot probably was attributable to *N*-acetylhistidine (No. 4 on Table 1 and Fig. 1) and the lower one could be histidine, carnosine or homocarnosine or a combination of the latter. Subsequent chromatography on Silica gel using solvents F and G, respectively, and visualization with Fast Red B (Fig. 4b) confirmed the position of *N*-acetylhistidine and suggested that the lower spot of Fig. 4a probably was homocarnosine (No. 14). Repeating the chromatography of Fig. 4b with added histidine (No. 7) in Fig. 4c and added carnosine (No. 13) in Fig. 4d clearly differentiated the chromogenic material from the latter two substances. However, when homocarnosine (No. 14) was added to the extract (Fig. 4e), no new spot appeared and there was an enlargement of the spot in the extract. From the above it was concluded that *N*-acetylhistidine and homocarnosine were present in the original extract at levels detectable by the procedures employed. Employing the quantitative procedures described above, it was estimated that trout brain contained 160 nmoles of *N*-acetylhistidine and 18 nmoles of homocarnosine per 100 mg of fresh weight.

Chromatography of extracts of brains of Pacific ocean blue perch (*Medialuna Calif.*) showed there to be a large amount of *N*-acetylhistidine and only a small amount of material that could be histidine, carnosine or homocarnosine. *N*-acetylhistidine and small amounts of histidine, carnosine and homocarnosine were found in frog brain (*Rana pipiens*) by the procedures described for trout brain. Only *N*-acetylhistidine was detectable in the brain of Pacific Rose fish (*Gadus*) and catfish (*Ictalurus lacustris*). We also found that *N*-acetylhistidine was present in brains of Pacific Sargo (*Anisotremus davidsoni*), Pacific Opaleye (*Girella nigricans*), and California fence lizard (*Sceloporus occidentalis*).

Application of methods to study of disposition of histidine and N-*acetylhistidine in Ehrlich ascites tumor cells.* In a previous study[6] it was found that histidine injected intraperitoneally into Ha/ICR Swiss mice with Ehrlich ascites tumor (diploid) was taken up avidly by the tumor cells. At the time of maximal uptake of a single dose, histidine was calculated to comprise approximately 1·5 to 2 per cent of the dry weight of the cells. Paper chromatography of the extracts did not show the presence of any diazo-positive substances other than histidine. In the present experiments, starting with the third day after the transplantation of 1×10^6 tumor cells, 100 μmoles of histidine (0·1 ml, pH 7·0) was injected daily intraperitoneally for 5 days and samples of tumor were taken at 24 hr after the last injection. Upon two-dimensional chromatography of perchloric acid extracts on Silica gel of separated tumor cells equivalent to 40 μl of packed cell volume, only histidine, itself, was detected (Fig. 5a). An experiment performed similarly with *N*-acetylhistidine likewise showed that *N*-acetylhistidine was the only detectable substance in the cell extracts (Fig. 5b). Thus, even

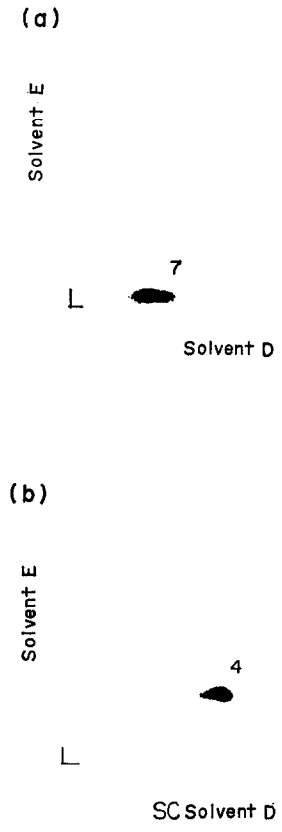

FIG. 5. Chromatogram of extract of Ehrlich ascites cells from animals receiving histidine intraperitoneally daily for 5 days as described in the text (a). Only histidine was detected by the diazo reaction. (b) is a similar experiment to that of (a) with the exception that *N*-acetylhistidine was injected. Only *N*-acetylhistidine was detected in this extract.

prolonged administration of the above substances did not lead to the accumulation of diazo-positive metabolites.

In another experiment four tumor-bearing mice were injected intraperitoneally on the seventh day after transplantation of 10^6 cells with 0·1 ml of a solution containing $1{\cdot}3 \times 10^6$ counts/min of U-^{14}C-histidine (spec. act. 50 mCi/m-mole) and cells were taken from each of two mice at 15 and 60 min, respectively, as well as from uninjected mice. Chromatography was performed on Silica gel (Fig. 6) with aliquots corresponding to 20 μl of packed cell volume employing added markers of homocarnosine, carnosine, histidine and *N*-acetylhistidine. Chromatograms were exposed for radioautography for 3 weeks. When U-^{14}C-histidine was added to the control cells after the addition of perchloric acid, radioactivity was detected only in histidine (Figs. 6a and b). The results in Figs. 6c–6f show that both at 15 or 60 min no radioactivity was detected in any substance other than histidine. Thus, the above results show clearly that, at most, very small amounts of histidine metabolites are present in Ehrlich ascites tumor cells at times when the intracellular concentrations of the amino acid are high.

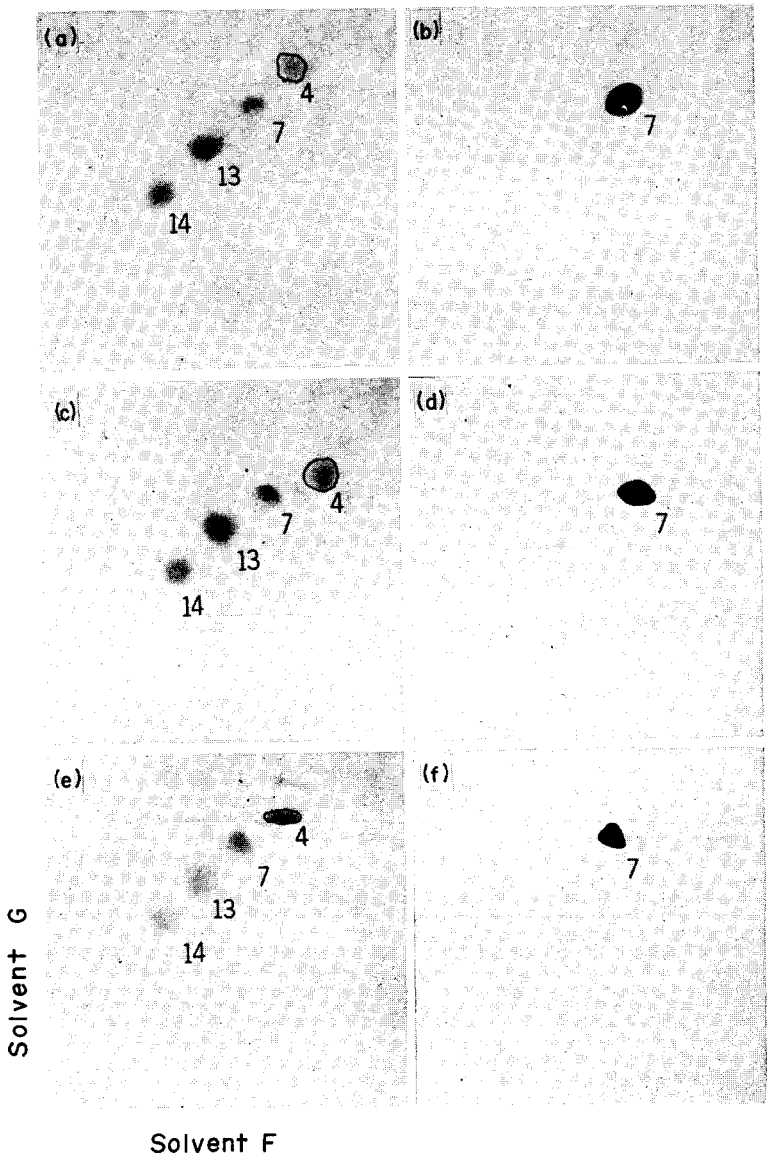

FIG. 6. Only histidine was detected by radioautography of TLC plates after the intraperitoneal injection U-^{14}C-histidine into mice bearing the Ehrlich ascites tumor. Figs. 6a and b are pictures of the sprayed chromatogram and radioautograph of the control samples respectively. Figs. 6c and d and 6e and f were obtained from extracts of tumor cells at 15 and 60 min, respectively, after injection of the isotope. See text for details.

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