THIN-LAYER CHROMATOGRAPHY OF SOME ^{131}I AND ^{125}I LABELLED IODOPYRIMIDINES AND RELATED NUCLEOSIDES

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(Received June 22nd, 1964)

INTRODUCTION

Iodinated pyrimidines and nucleosides labelled with radioiodine (^{131}I or ^{125}I) at a very high specific activity are of great interest in studies directed to metabolic chemotherapy of cancer, which are being actively pursued in many laboratories[1]. These investigations are concerned with compounds such as IUDR (5-iodo-deoxyuridine) which interfere specifically with the incorporation of thymidylic acid into DNA, or are able, to some extent, to substitute for thymidylic acid in the biosynthesis of DNA[2]. Other attempts to obtain antimetabolites[3] included studies of the 5-iodo-derivatives of uracil and uridine, and of cytosine and cytidine[4] labelled with radioiodine.

The preparation of labelled iodopyrimidines and nucleosides involves an isotopic exchange reaction in aqueous solution between the inactive iodinated molecules and the radioiodine as iodide ion[5]. A side reaction involving partial hydrolysis of nucleosides often takes place, which gives rise to corresponding pyrimidines. "Free" iodine, as I′, or I_2 is always present in the solutions of radioiodinated pyrimidines or nucleosides, both during the labelling process, because of an incomplete exchange reaction, and in the final products, because of the autoradiolysis. The routine production of labelled pyrimidines and nucleosides requires a rapid and selective method of analysis in order to control the labelling procedure, to measure the exchange yields and to check the purity and identity of the final products.

With this purpose in mind, the separation by thin-layer chromatography, of 5-iodouracil, 5-iodocytosine, related nucleosides and I′ was studied.

A large number of solvent systems were tested on silica gel G and cellulose G, using ascending chromatography, in order to develop a chromatographic method which yields a good resolution of the different components. Since the evaluation of the amounts of the labelled components in a sample is based on the measurement of the areas of the chromatographic peaks obtained by an automatic scanning of the plate and a data recording system, a sharp resolution of the chromatographic spots is an essential condition.

EXPERIMENTAL

Preparation of iodinated pyrimidines and nucleosides

5-Iodouracil and 5-iodocytosine were prepared, according to the method proposed by JOHNSON AND JOHNS[6], by direct iodination of uracil or cytosine in al-

kaline solution at 50–60°. The purity of the products was checked by U.V. spectrophotometry and paper chromatography.

5-Iododeoxyuridine and 5-iodouridine were prepared according to the method proposed by PRUSOFF[7]. A mixture of deoxyuridine (supplied from BDH), iodine, chloroform and nitric acid was refluxed for 2 h until needle crystals of IUDR appeared. The product was recrystallized from hot water and the purity was checked by U.V. spectrophotometry and chromatography. The same procedure was adopted for 5-iodouridine, but the refluxing was continued for 18 h. 5-Iodocytidine was prepared by a modification of the method described by CHANG[8] for the preparation of 5-iododeoxycytidine. Cytidine (100 mg), glacial acetic acid (0.3 ml), iodic acid (35 mg), iodine (60 mg), chloroform (0.1 ml) and water (0.1 ml) were gently warmed for 18 h at 40°. The precipitated white crystals of iodocytidine were washed with ether until all the unreacted iodine was extracted, and then dissolved in glacial acetic acid. Undissolved HIO_3 was discarded and iodocytidine was precipitated by ether and separated by centrifugation. A more detailed description of the preparation procedure and the results of the identity controls will be reported elsewhere[9].

The labelling of iodinated pyrimidines and nucleosides with either ^{131}I or ^{125}I was carried out in aqueous solution by means of an exchange reaction with the radioiodine as iodide ion, at 100° for iodouracil and iodocytosine, at 60° for iodouridine and iododeoxyuridine, and at 40° for iodocytidine.

Preparation of the chromatographic plates

Plates of mirror glass (thickness 3 mm, width 50 mm, length 200 mm) were coated with a 0.3 mm layer of silica gel G or cellulose G by means of a Chemetron apparatus.

Silica gel G plates were dried for 30 min at 110° and allowed to cool in a moisture-free chamber. Cellulose G plates were dried for 40 min at 50°. A sample of the solution to be examined (about 50 μl of a 0.1 % aqueous solution of iodinated labelled pyrimidines or nucleosides) corresponding to approximately 50 μC, was applied by means of a capillary pipette to the plate on a line 2.5 cm from the edge of the plate. The development was carried out by the ascending method in closed rectangular tanks saturated for 1 h with the appropriate solvent system and lined with filter paper for a good equilibration. The developed chromatograms were removed from the tanks, dried and then analyzed by an automatic scanning and recording device.

Scanning and recording system

The chromatographic plates were analyzed by a scanning and recording device, which has been described in detail elsewhere[10].

The plate is laid upon an aluminium slide which runs at a constant speed under a radiation detection unit (phototube Dumont 6292, 1.5 in. × 1.5 in. NaI(Tl) crystal) connected through a single channel analyzer with a recording apparatus (EKCO N 522 ratemeter and Speedomax G graphic recorder). In each experiment the shape and the position of the chromatographic spots was previously checked by autoradiography of the plates using Ferrania-X film, exposed for periods of up to 16 h. The film was protected from acidic materials on the plates by thin polyethylene foils. The R_F values of the single components were directly measured on the recorded chromatograms.

RESULTS AND DISCUSSION

The R_F values measured on silica gel and cellulose are listed respectively in Tables I and II. Each R_F value was at first measured for the pure separated compound and then checked again using mixtures.

The results in Tables I and II show that, with a suitable choice of the elution mixture and support, it is possible to attain very satisfactory separations.

TABLE I

R_F VALUES ON CELLULOSE G PLATES

	R_F							
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*
5-Iodouracil	0.83	0.77	0.80	0.79	0.71	0.85	0.84	0.83
5-Iodouridine	0.86	0.54	0.59	0.58	0.48	0.85	0.61	0.57
5-Iododeoxyuridine	0.90	0.74	0.78	0.75	0.67	0.74	0.81	0.80
5-Iodocytosine	0.47	0.48	0.48	0.52	0.45	0.61	0.57	0.45
5-Iodocytidine	0.66	0.30	0.13	0.14	0.24	0.62	0.32	0.30
Iodide	1.00	0.35	0.37	0.41	0.91	0.92	0.48	0.59
Time of run (in min)	60	240	240	240	60	60	180	180

a = water; b = *n*-butanol saturated with water; c = *n*-butanol saturated with 0.1 *N* H_3BO_3; d = *n*-butanol saturated with 1 *N* H_3BO_3; e = saturated solution H_3BO_3 in water; f = 0.1 *N* HCOOH; g = *n*-butanol saturated with 0.1 *N* HCOOH; h = *n*-butanol saturated with 0.1 *N* NH_4OH.

When the distribution of the radioactive iodine between the different components is to be evaluated by scanning, thin-layer radiochromatography appears to be very useful for the analysis of mixtures such as iodouridine, iodouracil and iodide (elution mixture (c) on cellulose, (e) on silica gel, as shown in Fig. 1) and iodocytidine, iodocytosine and iodide (elution mixtures (c) and (e) on cellulose, (e) on silica gel as

TABLE II

R_F VALUES ON SILICA GEL G PLATES

	R_F							
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*
5-Iodouracil	0.59	0.70	0.69	0.74	0.62	0.56	0.68	0.45
5-Iodouridine	0.72	0.47	0.47	0.50	0.52	0.65	0.47	0.45
5-Iododeoxyuridine	0.57	0.65	0.65	0.70	0.60	0.50	0.63	0.42
5-Iodocytosine	0.58	0.49	0.49	0.52	0.58	0.57	0.53	0.40
5-Iodocytidine	0.68	0.27	0.27	0.29	0.73	0.64	0.42	0.42
Iodide	0.91	0.19	0.18	0.19	0.90	0.81	0.24	0.22
Time of run (in min)	120	300	300	300	120	120	240	240

a = water; b = *n*-butanol saturated with water; *c* = *n*-butanol saturated with 0.1 *N* H_3BO_3; d = *n*-butanol saturated with 1 *N* H_3BO_3; e = saturated solution H_3BO_3 in water; f = 0.1 *N* HCOOH; g = *n*-butanol saturated with 0.1 *N* HCOOH; h = *n*-butanol saturated with 0.1 *N* NH_4OH.

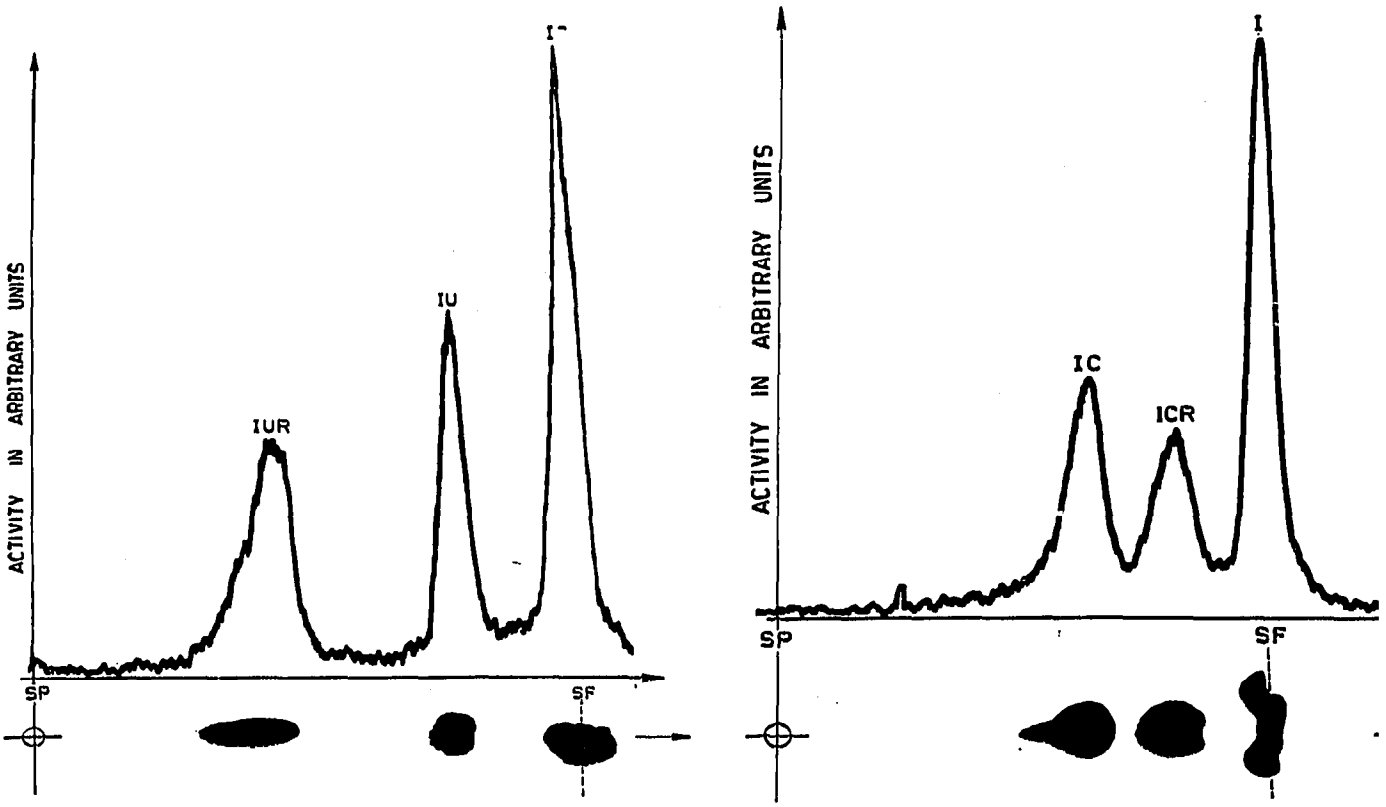

Fig. 1. Scan and autoradiograph of silica gel G plate on which ^{131}I iodouracil (IU), ^{131}I iodouridine (IUR) and iodide ions were separated by eluting with saturated H_3BO_3. Time of run: 60 min.

Fig. 2. Scan and autoradiograph of cellulose G plate on which ^{131}I iodocytosine (IC), ^{131}I iodocytidine (ICR) and iodide ions were separated by eluting with saturated H_3BO_3. Time of run: 120 min.

shown in Fig. 2). Satisfactory separations are obtained for mixtures of the 3 nucleosides and separations of mixtures of labelled ^{131}I iodouridine (IUR), IUDR and ^{131}I iodocytidine (ICR) are shown in Figs. 3 and 4; the iodide ions come from the autoradiolysis of the labelled molecules which were stored for 20 days before analysis. As is shown by autoradiography in Fig. 3, sharp separation can be attained between the spots of ICR, IUR and IUDR; iodide ions interfere with IUR, making the quantitative estimation of this nucleoside more difficult. It must be kept in mind that an improved separation of the recorded peaks could be obtained by lessening the background effect by using a narrower slit with the detection unit.

With a saturated solution of boric acid as solvent, a good resolution of the spots can be attained for IUR, IUDR, ICR and iodide ions, as is shown by the autoradiography reported in Fig. 4.

Since "*in vivo*" degradation of a labelled nucleoside gives rise normally to a pyrimidine and to "free" iodine as I', this method should be very useful in the study of the biological behaviour of these products, when the analysis of many samples of blood or urine is required. In this case previous desalting of the samples to be analyzed is probably not necessary.

Assays carried out by the authors showed that NaCl in isotonic concentration in the mixture to be analyzed does not induce substantial modifications in the chromatographic separation.

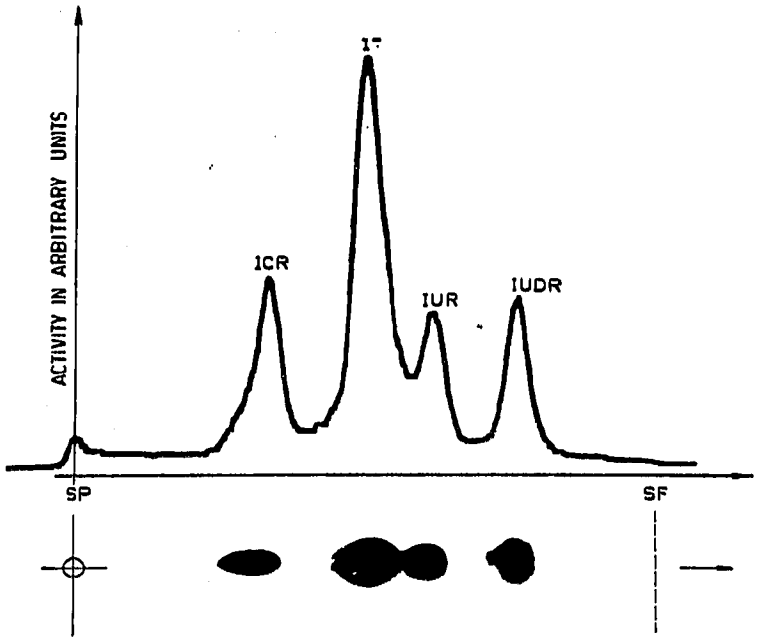

Fig. 3. Scan and autoradiograph of a silica gel G plate on which ^{131}I iodocytidine (ICR), ^{131}I iodouridine (IUR) and ^{131}I iododeoxyuridine (IUDR) were separated by eluting with *n*-butanol, saturated with 1 *N* H_3BO_3. Time of run: 240 min.

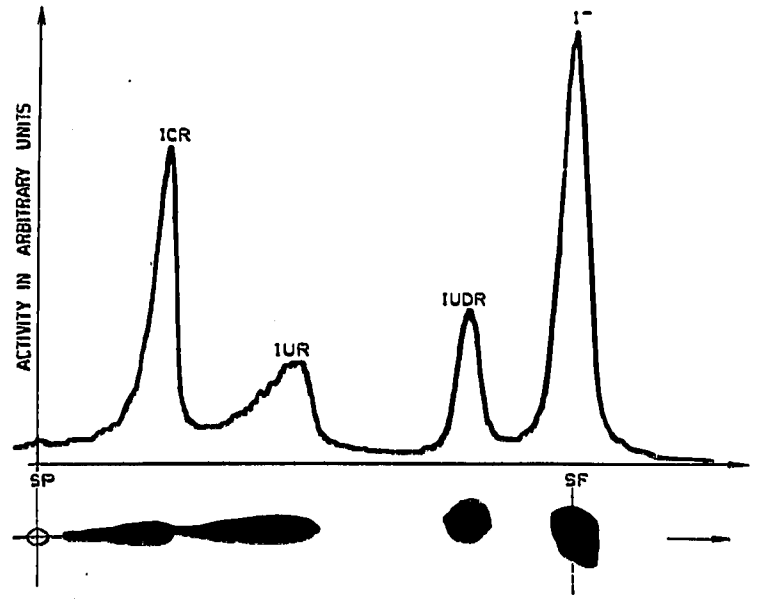

Fig. 4. Scan and autoradiograph of a silica gel G plate on which ^{131}I iodocytidine (ICR), ^{131}I iodouridine (IUR), and ^{131}I iododeoxyuridine (IUDR) were separated by eluting with saturated H_3BO_3. Time of run: 60 min.

SUMMARY

The separation of some radioiodinated pyrimidines and nucleosides of biological interest was studied by thin-layer chromatography.

R_F values of iodouracil, iodocytosine, iodouridine, iododeoxyuridine and iodocytidine on layers of silica gel G and cellulose G were measured for a number of

solvents: *n*-butanol saturated with water, water, *n*-butanol saturated with 0.1 *N* H_3BO_3, *n*-butanol saturated with 1 *N* H_3BO_3, a saturated solution of H_3BO_3 in water, 0.1 *N* HCOOH, *n*-butanol saturated with 0.1 *N* HCOOH and *n*-butanol saturated with 0.1 *N* NH_4OH were used. With a proper choice of elution system and support, a very good resolution of the single spots can be attained and the relative percentages of the labelled components in a sample can be evaluated with a negligible error by radioactive scanning of the chromatographic plate.

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