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STUDIES IN THE MOUSE OF THE PHARMACOLOGY OF 5-IODODEOXYURIDINE, AN ANALOGUE OF THYMIDINE

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Abstract—5-Iododeoxyuridine labeled with I^{131} or tritium was administered to normal and tumor-bearing mice, and a study was made of its metabolism, tissue distribution and excretion. The main pathway for degradation of IUdR involved a cleavage to 5-iodouracil and subsequent dehalogenation with the formation of uracil and inorganic iodide. Considerable iodination of non-nucleic acid derivatives was observed in the protein fractions of cells. The occurrence of iodinated derivatives of acyclic products of iodouracil catabolism was not demonstrated, although these probably were formed as a result of direct degradation. The acute LD_{50} of IUdR in mice was 2·5 g/kg. The LD_{50} for IUdR upon chronic administration intraperitoneally for 13 days was 318 mg/kg; this toxicity could be prevented completely by the prior administration of thymidine.

5-IODODEOXYURIDINE (IUdR),[1] an analogue of thymidine, is an effective inhibitor of the growth of several neoplasms in mice[2] and a competitive antagonist of the utilization of thymidine in microbial[1, 3] and mammalian[1, 2, 4, 5] systems. Studies of the mechanism of action of the analogue in mouse Ehrlich ascites tumor cells[1, 6] disclosed an inhibition of the utilization of precursors of pyrimidines for the biosynthesis of DNA-thymine, but not for RNA-uracil and -cytosine or DNA-cytosine. Because IUdR was utilized, in competition with thymidine, for which it substituted in the biosynthesis of DNA[4, 5, 7, 8, 9] and since it inhibited the incorporation of thymidylic acid into DNA in a cell-free system;[10] it is suggested that the site of inhibition is at the mono- or tri-phosphate level.

The present report is a study of the metabolism, tissue distribution, excretion and toxicity of IUdR in the mouse.

MATERIALS AND METHODS

Preparation of compounds. IUdR was synthesized by the procedure described previously[1], and I^{131}-labeled material was prepared by a modification of the method used for the synthesis of 5-iodouridine-I^{131}.[11] Tritiated IUdR was prepared from deoxyuridine labeled in position-5 and -6 with tritium.

Preparation and analysis of tissues. IUdR-I^{131} was administered intraperitoneally to normal mice and to mice* bearing the L-5178-Y lymphoma.† The tissues were removed at various intervals of time; these were either dissolved in hot 6N NaOH and adjusted to a constant volume or were fractionated by the Schneider modification[12] of the Schmidt–Thannhauser procedure[13] into cold acid-soluble, combined nucleic acids,

* Mice bearing L-5178-Y were AKR × DBA/2 F_1 hybrids, obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

† The Y-strain of leukemia L-5178 was developed by G. A. Fischer and J. J. Jaffe from a single cell, isolated *in vitro* and grown initially in a medium which has been described by G. A. Fischer and A. D. Welch, Science, **126**, 1018 (1957). The original donor mice carrying L-5178 were kindly provided by L. W. Law of the National Cancer Institute.

and protein fractions. The protein fraction was dissolved in warm 6N NaOH and each of the three fractions from each tissue was brought to a common volume. The concentration of radioactive iodine was determined using a well-type gamma scintillation counter and the concentration of tritium either by using a liquid phosphor counter or by plating aliquots on stainless steel planchets and counting in a windowless gas-flow Geiger counter.

RESULTS AND DISCUSSION

Organ distribution

The amount of radioactivity in a specific tissue and the relative distribution varied considerably between different experiments and may be a result of different dosage

TABLE 1. TISSUE DISTRIBUTION OF RADIOACTIVITY IN NORMAL AND TUMOR-BEARING MICE INJECTED INTRAPERITONEALLY WITH A SINGLE DOSE OF RADIOACTIVE SODIUM IODIDE OR 5-IODODEOXYURIDINE*

Organ	Iododeoxyuridine				Sodium Iodide	
	0·5 hr		21 hr		21 hr	
	Tumor-bearing	Control	Tumor-bearing	Control	Tumor-bearing	Control
Tumor	21,300	—	3,650	—	200	—
Kidney	49,300	38,400	397	200	183	89
Spleen	26,000	26,600	4,480	3,180	25	32
Lymph node	20,600	23,000	3,400	2,000	13	290
Intestine	30,800	21,400	4,000	2,950	120	35
Liver	29,800	27,500	350	174	116	56
Heart	19,200	17,400	295	225	None	17
Lung	21,000	25,400	824	355	114	20
Thyroid	17,100	17,200	1,000	1,000	2,800	1,630
Testes	15,000	14,400	735	740	130	41
Brain	2,600	2,700	None	50	None	None
Thymus	16,100	19,600	3,420	4,400	320	105
Stomach	49,200	42,600	2,180	1,860	504	350
Skin	15,600	21,000	1,800	720	1,050	220
Muscle	12,200	13,900	311	145	130	167
Bone marrow†	2,600	2,900	1,400	776	50	32

* Both NaI and IUdR were labeled with I^{131}. Two groups of three mice each received intraperitoneally a single injection of 5 μmoles $4{\cdot}0 \times 10^6$ counts/min per μmole of the indicated compound. After the appropriate time the tissues were removed, weighed and dissolved or suspended in KOH (6 N; to 10 ml). The concentration of radioactivity is given in counts/min per gram of wet weight of tissue.

† Counts/min per tissue, rather than counts/min per g.

regimens employed in the administration of radioactive IUdR-I^{131}, as well as variations in the time of sacrifice of the mice. In Table 1 is shown the tissue distribution of radioactivity observed when tumor and non-tumor bearing mice* received a single injection of NaI^{131} (5 μmoles) or IUdR-I^{131} (5 μmoles) and were sacrificed after 0·5 or 21 hr. There was no striking difference between the tissues of normal mice and those bearing tumors in the localization of radioactivity derived from either compound and such differences which were observed may be insignificant because of the relatively small

* Mice used in studies of the toxicity and tissue distribution of IUdR-I^{131} were Swiss females, obtained from Millerton Research Farm, Millerton, N.Y.

number of animals used. The concentration of radioactivity in the thyroid tissue was 14-fold in excess of the amount present in tumor tissue in those animals injected with NaI^{131}, whereas in mice treated with IUdR-I^{131} this relationship between the tissues was reversed; thus, the tumor had a 3-fold excess of radioactivity, as compared with that of the thyroid glands. The latter tissue had an appreciable concentration of radioactivity, a finding which indicated that a considerable amount of breakdown of IUdR to iodide occurred; this catabolic degradation was confirmed subsequently by experiment.

In Table 2 is shown the distribution observed when two groups of mice each bearing the L-5178-Y lymphoma received intraperitoneal injections of IUdR-I^{131} (10 μmoles) and were sacrificed 2 and 24 hr later, respectively. Consideration of the data of Tables 1, 2 and 3 indicates that there was a rapid drop in the tissue concentration of radioactivity within 2 hr of the time of administration of IUdR, and that this decrease was followed by a relatively slow rate of disappearance of radioactivity during the succeeding 48 hr. There was considerable retention of radioactivity even after 24 hr and the decrease in concentration of radioactivity in the tumor at 24 hr following treatment was only to about 50 per cent of the amount present 2 hr following the administration of the analogue (Table 2).

TABLE 2. DISTRIBUTION OF RADIOACTIVITY IN THE ACID-SOLUBLE, NUCLEIC ACID AND PROTEIN FRACTIONS OF TISSUES FROM MICE BEARING THE 5178-Y LYMPHOMA FOLLOWING INJECTION OF A SINGLE DOSE OF RADIOACTIVE 5-IODODEOXYURIDINE*

Time of Sacrifice	Tissue	Wet wt. (g)	Concentration of radioactivity†		
			Acid-soluble	Nucleic acid	Protein
2 hr	Tumor	0·61	600	4,200	4,200
	Liver	3·80	100	560	314
	Spleen	0·34	3,300	2,500	3,900
	Kidney	1·07	40	2,550	980
	Stomach	0·59	1,600	430	300
	Intestine	8·30	730	73	35
	Heart	0·56	427	400	197
	Bone marrow‡	—	74	680	970
24 hr	Tumor	0·83	None	1,520	1,860
	Liver	4·04	30	380	218
	Spleen	0·50	710	1,000	2,200
	Kidney	1·13	20	650	440
	Stomach	0·52	55	173	173
	Intestine	7·68	80	60	37
	Heart	0·50	100	400	290
	Bone marrow‡	—	60	70	440

* Six mice were injected intraperitoneally with radioactive iododeoxyuridine labeled with I^{131} (10 μmoles; $6{\cdot}1 \times 10^5$ counts/min per μmole). Groups of three mice were sacrificed 2 and 24 hr later by severing the spinal cord and the tissues were removed rapidly, weighed and frozen in a bath of alcohol and dry-ice. The combined organs from each group of mice were homogenized in ice-cold trichloroacetic acid (TCA) (5 per cent; 25 ml) for 3 min. The residue after centrifugation was extracted two additional times with cold TCA, prior to extraction with hot TCA (5 per cent; 25 ml; 90°) for 30 min. The residue, composed primarily of protein, was dissolved in warm alkali.

† Values are expressed in counts/min per g of wet wt. of tissue.

‡ Tissue included bone and the values given are for the total content of radioactivity in counts/min.

TABLE 3. DISTRIBUTION OF RADIOACTIVITY IN THE ACID-SOLUBLE, NUCLEIC ACID AND PROTEIN FRACTIONS OF TISSUES FROM MICE BEARING THE 5178-Y LYMPHOMA FOLLOWING THE INJECTION OF RADIOACTIVE SODIUM IODIDE OR 5-IODODEOXYURIDINE*

Compound injected	Tissue	Wet wt. (g)	Concentration of radioactivity†			
			24 hr			
			Acid-soluble	Nucleic acid	Protein	Nucleic acid: Protein
Sodium Iodide-I^{131}	Tumor	1·67	None	None	12	0·0
	Liver	4·00	None	10	76	0·13
	Spleen	0·55	None	None	88	0·0
	Kidney	0·94	None	29	188	0·15
	Stomach	1·10	146	18	99	0·18
	Intestine	8·9	None	9	83	0·11
	Lung	0·58	None	None	59	0·0
	Thyroid	0·46	None	None	16	0·0
	Heart	0·43	None	None	None	—
Iododeoxy-uridine-I^{131} plus NaI	Tumor	0·90	None	391	300	1·3
	Liver	4·40	None	30	59	0·5
	Spleen	0·45	None	460	292	1·6
	Kidney	0·91	None	40	22	1·8
	Stomach	0·67	None	204	163	1·3
	Intestine	8·62	None	142	342	0·4
	Lung	0·55	None	285	157	1·8
	Thyroid	0·47	None	161	84	1·9
	Heart	0·34	None	None	None	—
				48 hr		
Sodium Iodide-I^{131}	Tumor	2·11	None	None	None	—
	Liver	4·61	None	None	32	—
	Spleen	0·54	None	None	None	—
	Kidney	0·95	None	None	56	—
	Stomach	0·49	None	None	None	—
	Intestine	8·5	None	None	10	—
	Lung	0·52	None	None	None	—
	Thyroid	0·41	None	None	116	—
	Heart	0·44	None	None	70	—
Iododeoxy-uridine-I^{131} plus NaI	Tumor	1·62	None	87	62	1·4
	Liver	4·10	None	17	16	1·0
	Spleen	0·42	None	170	130	1·3
	Kidney	0·93	None	24	59	0·4
	Stomach	0·57	None	53	96	0·6
	Intestine	7·50	None	9	27	0·3
	Lung	0·47	None	55	49	1·1
	Thyroid	0·55	None	None	40	—
	Heart	0·51	None	None	39	—

* Six mice were injected intraperitoneally 4 times during a 3-day period with NaI^{131} ($1·5 \times 10^5$ counts/min) and another group of 6 mice with $IUdR\text{-}I^{131}$ (5μmoles; $1·2 \times 10^5$ counts/min) plus non-radioactive sodium iodide (5 mg). Twenty-four and 48 hr following the last injection the animals were sacrificed and the tissues analyzed in the manner described in Table 2.

† Values are expressed in counts/min per g of wet wt. of tissue.

Fractionation of tissues

The tissues of mice which had received $IUdR\text{-}I^{131}$ were fractionated into cold acid-soluble, combined nucleic acids, and protein fractions, and their content of radioactivity was determined (Table 2). The radioactivity in the acid-soluble fraction derived

from the tumor tissues completely disappeared within 24 hr, while that of other tissues showed a marked decrease. The lowering of the concentration of radioactivity in the protein and nucleic acid fractions ranged from 25–75 per cent.

Although incorporation of a pyrimidine into DNA has been demonstrated by many investigators to lead to a relatively stable substance, i.e., with but little "turn-over", Zamenhof *et al*[14] have observed that halogenated pyrimidines may undergo an exchange reaction in the DNA of micro-organisms. Whether the observed decrease in the radioactivity of the nucleic acid fraction was a reflection of such a phenomenon (exchange with thymine) or of the loss of non-nucleic acid components associated with this fraction, either physiologically or as a result of the method of fractionation employed, is unknown and should be investigated. It is known that hot trichloroacetic acid (TCA) does produce partial hydrolysis of proteins and hence the "nucleic acid" fraction does contain amino acids, of which some may contain radioactive iodine. However, it has been shown that IUdR-I^{131} is incorporated into DNA[4, 5, 7, 8, 9] but what percentage of the observed radioactivity in this fraction represented *bona fide* radioactive nucleic acid has not yet been determined.

The following study was conducted in an attempt to minimize non-specific absorption of radioactive iodide, as well as the formation of non-nucleic acid reaction products such as iodinated amino acids. Non-radioactive sodium iodide (5 mg) was administered together with IUdR-I^{131} (5 μmoles) to two groups of mice which were sacrificed 24 or 48 hr after the last of 4 injections. Two additional groups received NaI^{131} alone and were sacrificed at the same intervals of time. The results are shown in Table 3. The radioactivity in the NaI^{131}-treated animals was incorporated primarily into the protein fraction and the amount of radioactivity present in the nucleic acid fraction disappeared completely within 48 hr. Whereas the administration of NaI^{131} alone resulted (after 24 hr) in a ratio of the radioactivity in the nucleic acid to protein fraction of less than 0·2, the concentration of radioactivity in the animals treated with IUdR-I^{131} was generally in favor of the nucleic acid fraction. In agreement with the previous experiment (Table 2), there was a marked decrease in the content of radioactivity in the nucleic acid fraction.

Metabolic derivatives of IUdR

To obtain information concerning the metabolism of IUdR the cold acid-soluble fraction of various tissues, as well as the urinary excretion products from mice with radioactive IUdR, were studied.

Twenty-eight young adult (22–25 g) Swiss female mice bearing a heavy population of Ehrlich ascites tumor cells (each mouse received about 5×10^7 cells intraperitoneally 4 days previously) were injected with 12 μmoles of IUdR-I^{131} divided into 4 doses given at 12 hr intervals. Three hours following the last injection the animals were sacrificed and the ascites tumor cells were collected by centrifugation with a total yield of 24·7 g wet-weight. The cells were extracted with ice-cold $HClO_4$ (0·5N; 250 ml) and following neutralization with KOH the formed $KClO_4$ was removed by filtration. The filtrate was adjusted to pH 9·8 and passed through a Dowex-1 formate column (2·5 cm $\times$ 30 cm). The ion exchange chromatogram obtained by gradient elution is shown in Fig. 1. Two radioactive areas, A and B, were obtained. Fraction A was mixed with non-radioactive iodouracil and iododeoxyuridine and aliquots were chromatographed on paper using the ethyl acetate–phosphate buffer system,[15] as well

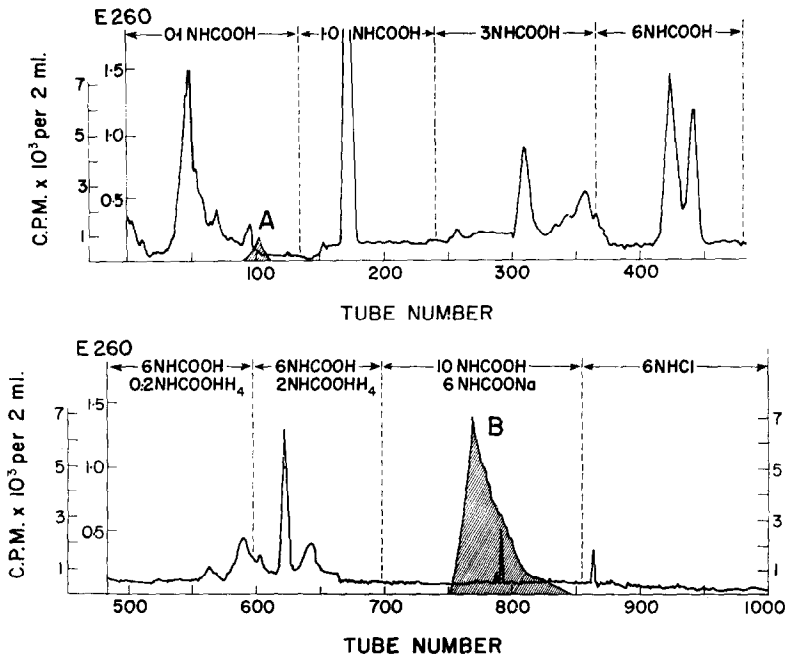

FIG. 1. Elution pattern of metabolic derivatives of IUdR-I^{131} from the cold acid-soluble fraction of Ehrlich ascites tumor cells (see text for details). Shaded area indicates radioactivity.

as on a Dowex-1 formate column (Fig. 2). Under both conditions the radioactivity coincided with that of iodouracil. Fraction B contained more than 95 per cent of the radioactivity in the acid-soluble fraction and was identified as iodide ion (see below).

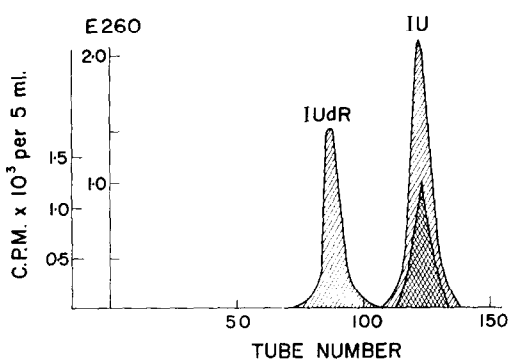

FIG. 2. Identification of fraction A (Fig. 1). Radioactive fraction A was mixed with non-radioactive 5-iodouracil and 5-iododeoxyuridine adsorbed on a Dowex-1 formate column (30 cm × 2·5 cm) and eluted by gradient elution with 0·1N HCOOH according to the method of Hurlbert *et al.*[22] Each tube contained 5 ml of effluent (see text for details). Cross lined area indicates radioactivity.

Rate of excretion of IUdR

The rate of excretion of radioactivity following the intraperitoneal injection of a single dose of IUdR-I^{131} is shown in Table 4. Within 4 hr approximately 74 per cent

of the radioactivity administered had appeared in the urine, and maximum excretion (91 per cent) was observed within 24 hr.

Urinary metabolic derivatives of IUdR

Each of four mice was injected with IUdR-I^{131} (5 μmoles; $4{\cdot}2 \times 10^6$ counts/min; 1.0 ml) and the urine was collected in the manner described in the footnotes of Table 4.

TABLE 4. CUMULATIVE EXCRETION OF RADIOACTIVITY IN THE URINE FOLLOWING THE INTRAPERITONEAL ADMINISTRATION OF IUdR-^{131} TO MICE*

Time (hr)	Total radioactivity (counts/min $\times 10^5$)	Per cent of administered dose excreted†
4	6·1 6·4 6·2	73·8
8	6·5 6·4 6·8	77·8
12	6·8 6·3 8·1	83·9
24	7·9 7·4 7·6	90·5
48	8·2 7·8 7·0	90·8

* Fifteen mice were placed individually in glass beakers and the urine was collected on filter paper protected by a wire screen. Each mouse received a single injection of IUdR-I^{131} (5 μmoles; $8{\cdot}4 \times 10^5$ counts/min; 1·0 ml) and 3 animals were sacrificed at each of the indicated times. The radioactivity was eluted from the filter paper with water in a Waring blendor, filtered, and an aliquot counted in a gamma scintillometer.

† Average for the group of 3 mice.

The pH of the urine collected during 24 hr was adjusted to 10·5, passed through a Dowex-1 formate column (200–400 mesh; 30 cm $\times$ 2·5 cm) and the latter was subjected to gradient elution with formic acid (2N), followed by formic acid (6N), NaCl (6N), and KI (6N). Three major radioactive peaks were obtained (Table 5). Iododeoxyuridine and iodouracil were eluted by low acid concentration; however, the free iodide appeared in the eluate only after KI was passed through the column. Following the administration of IUdR-I^{131}, radioactive iodide was the major radioactive substance present in the urine; in fact, it accounted for 83 per cent of the radioactivity present in the urine. Identification of this radioactive substance as iodide was made by the addition of concentrated HNO_3 (1 ml) to a 5 ml aliquot of the eluate, followed by continuous extraction with ether. Another aliquot was treated similarly with nitric acid, but the extraction was done with $CHCl_3$. The amount of radioactivity which appeared in the combined organic phases accounted for 97 per cent of the radioactivity present originally. This evidence, in addition to the failure of 6N NaCl, but the successful use of 6N KI, in the elution of the radioactive material from the Dowex-1 column, was

interpreted as identification of free iodide. The major non-radioactive substance was identified as uracil on the basis of its UV-spectra in acid and alkali, as well as its mobility on paper using several solvent systems. Furthermore, experiments to be described below with IUdR-6-H^3, unequivocally prove that the uracil was a metabolic derivative of IUdR.

TABLE 5. EXCRETION OF SOME METABOLIC DERIVATIVES OF 5-IODODEOXYURIDINE IN THE URINE OF MICE FOLLOWING THE ADMINISTRATION OF A SINGLE DOSE OF 5-IODODEOXYURIDINE-I^{131}*

Radioactive metabolic product	Per cent in urine
Iododeoxyuridine	6·6
Iodouracil	3·8
Iodide	83·2

* Each mouse was injected with 5 μmoles of IUdR-I^{131} and the urine was collected and measured as described in Table 4.

The following experiment was performed because the urine in the above experiment had been collected on filter paper with the concomitant possibility that the formation of iodide may have originated as a result of bacterial degradation following excretion, rather than being a *bona fide* metabolic derivative. Hence, when mice bearing the Ehrlich ascites tumor cells were injected with IUdR-I^{131} (see above and Fig. 1), the urine was collected in a tube suspended in a dry ice–alcohol bath. The pH of the urine was adjusted to 10·5, filtered, adsorbed on a Dowex-1 formate column ($\times$ 8; 200–400 mesh; 30 cm $\times$ 2·5 cm) and subjected to gradient elution (Fig. 3). A minimum of 7 radioactive peaks was observed; of these 7, 3 were identified as IUdR, iodouracil and iodide, respectively. The first two radioactive areas were re-chromatographed separately on Dowex-1 formate columns and the UV-spectra in acid and alkali were identical to those of IUdR[1] and iodouracil, respectively.

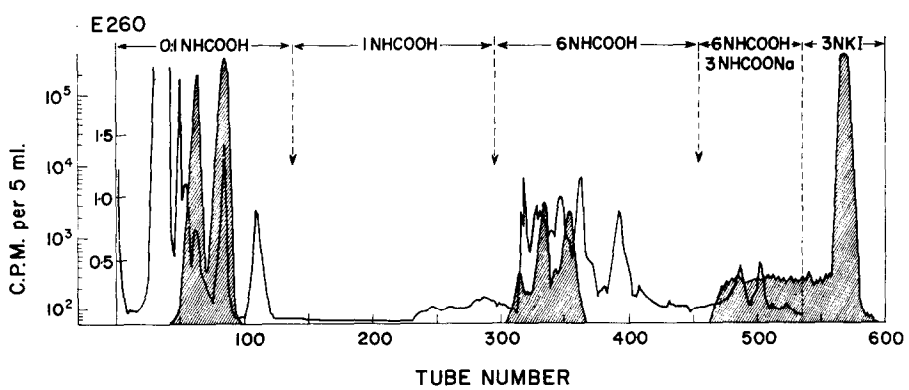

FIG 3. Elution pattern of urine from mice injected with IUdR-I^{131} (see text for details). Shaded area indicates radioactivity.

Metabolism of tritiated IUdR

Because large amounts of free iodide formed following the administration of IUdR-I^{131} under *in vivo* and *in vitro* conditions, it was of interest to identify the deiodinated reaction products. Accordingly, 10 mice were injected with IUdR-6-H^3 (3·16 μmoles; 3 μc) and the urine was collected in a tube suspended in an alcohol–dry ice bath. The urine was adjusted to pH 10·8 with NH_4OH, absorbed on a Dowex-1 formate column (30 cm $\times$ 2·5 cm), and subjected to gradient elution (Fig. 4); seven radioactive areas were observed. The first radioactive peak corresponded to the major UV-absorbing area and this area following reduction in volume was subjected to paper chromatography in three solvent systems (*iso*propanol-HCl,[16] butanol–H_2O,[17] and ethyl acetate–phosphate buffer (pH 6·0[15]). Two radioactive and two Uv-absorbing areas were identified, and in all three solvent systems one of the Uv-absorbing areas possessed radioactivity. Neither area contained a deoxyribose group, as evidenced by a negative reaction for this sugar using the method of Stumpf.[18] The Uv-area which was radioactive had the spectral properties characteristic of uracil, and, in the three paper chromatograms, this material had an R_F value which was identical to that of uracil. The other radioactive area, which contained the larger proportion of radioactivity, did not coincide with the other Uv-absorbing area. On the basis of paper chromatography in several solvent systems this major radioactive area was not dihydrouracil, ureidopropionic acid or β-alanine. The specific activity of the uracil isolated was 30,800 counts/min per μmole and hence represented a 32-fold dilution of the specific activity of the administered IUdR-H^3. Whether iododeoxyuridine resulted in an inhibition of the degradation of uracil, in a manner comparable to that reported for azathymine,[19] should be investigated.

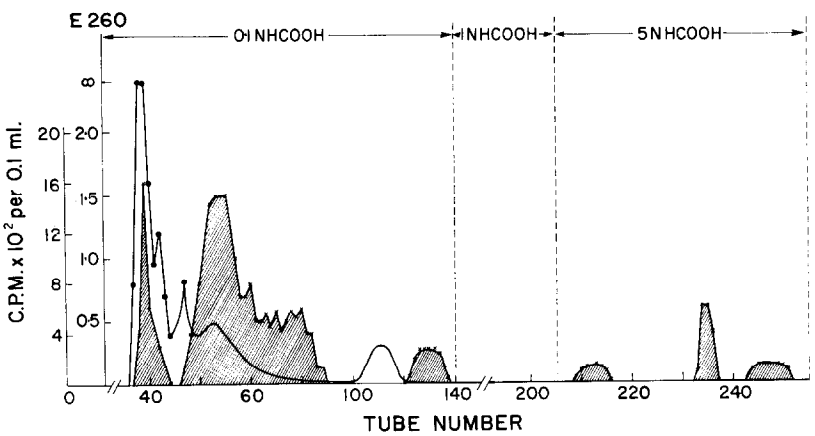

FIG. 4. Elution pattern of urine from mice injected with IUdR-H^3 (see text for details). Shaded area indicates radioactivity.

Toxicity of iododeoxyuridine

Acute toxicity from IUdR in Swiss mice resulted from the intraperitoneal administration of quantities in excess of 1·25 g/kg (Table 6); the LD_{50} was approximately 2·5 g/kg.

TABLE 6. ACUTE TOXICITY OF 5-IODODEOXYURIDINE IN MICE*

Amount of IUdR administered (g/kg)	Number of mice	Number of dead			
		24 hr	48 hr	72 hr	96 hr
None	10	None	None	None	None
2·5	13	8	None	None	None
2·00	11	1	1	None	None
1·25	10	None	None	1	None

*IUdR was suspended in 0·5 per cent carboxymethylcellulose in saline and each mouse received a single injection intraperitoneally (1·0 ml). The control mice were injected with the suspending media (1·0 ml).

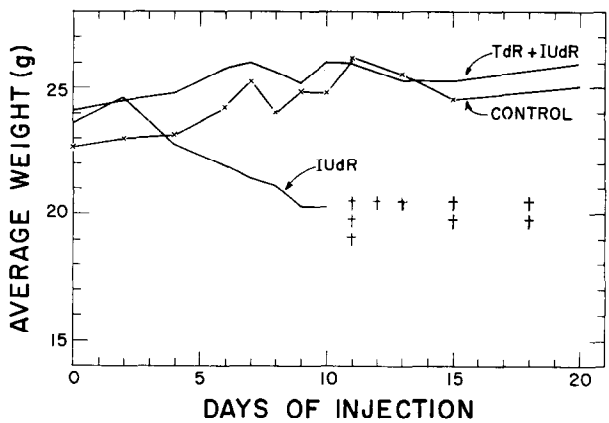

FIG. 5. Chronic toxicity of 5-iododeoxyuridine in mice and prevention by thymidine (see text for details).

In Fig. 5 is shown the results of a study of the chronic toxicity of IUdR in Swiss mice and the successful prevention of this toxicity by the prior administration of thymidine. Four groups of 10 mice were given daily intraperitoneal injections of 3, 6, 9 or 18 μmoles, respectively, of IUdR dissolved in water, while 4 additional groups were given 100 μmoles of thymidine prior to the administration of the stated amounts of IUdR; the control group was injected with saline. Only in the group which received the highest dose of IUdR, without thymidine, was loss of weight observed within a few days; on the 11th day of this dosage regimen 3 mice died. Half of the animals which received the high level of IUdR (18 μmoles per day) were dead by the 13th day and all had died by the 18th day. Thymidine prevented completely not only the lethal effects of IUdR, but also the loss of weight.

In Fig. 6 is shown a postulated scheme for the metabolism of IUdR. It is significant that no evidence for the formation of a primary dehalogenation product of IUdR was observed. If deoxyuridine were formed it must be very rapidly cleaved by nucleosidase to form free uracil. Because both iodouracil and uracil were observed the initial degradation step most probably was a direct cleavage of the iodinated deoxyribonucleoside with the formation of iodouracil.

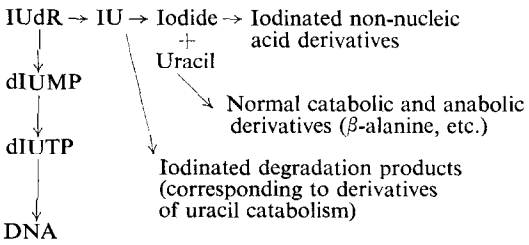

FIG. 6. Proposed scheme for the anabolism and catabolism of 5-iododeoxyuridine.

Studies of the metabolism of a related compound, 5-bromouracil, in human as well as other mammalian species, have been made[20, 21] in which reduction was reported of the double bond in the 5,6-position of the pyrimidine ring, prior to dehalogenation and the formation of uracil. That a similar reduction of 5-iodouracil occurred in the mouse would appear probable, because of the similar characteristics of iodo- and bromo-uracil; however, definitive evidence for this has not as yet been obtained. Comparison of the metabolic derivatives of radioactive IUdR labeled with I^{131} and with tritium indicated a minimum of 4 products which contained both labels. Whether some of these correspond to acyclic iodinated degradation products of uracil remains to be determined.

Because of the many metabolic derivatives of IUdR formed in the mouse, it is difficult to relate with certainty the observed toxicity to either a single compound or the interference with a single metabolic reaction. Whether the toxic substances even contain iodine, cannot yet be stated positively. However, the prevention of the toxicity of IUdR by thymidine is strongly indicative of the presumptive mechanism of action. Normally, IUdR is rapidly metabolized and excreted in the urine, and other studies[6] have shown that cells utilize thymidine about 40-fold more effectively than IUdR, for the biosynthesis of DNA *in vivo*. Hence, one may visualize thymidine as saturating the sensitive enzyme surface for a sufficiently long period to permit the intracellular concentration of IUdR to be lowered, by catabolism and excretion, to a non-toxic level. The concentration of IUdR required to produce toxicity was very critical, since a decrease in the amount, administered chronically to the mouse, from 18 μmoles to 9 μmoles resulted in a striking inability of the analogue to produce any manifestations of toxicity.

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