Iodothiouracil as a Melanoma Localizing Agent

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Thiouracil and various derivatives are selectively incorporated into the melanin pigment of melanomas during biosynthesis by serving as false melanin precursors. Using the transplantable Harding-Passey melanoma carried in BALB/c mice, we have extended our previous studies with sulfur-35 (^{35}S) thiouracil. The persistence of high levels of [^{35}S]thiouracil in tumor for periods of up to 2 wk has been demonstrated; during this time the drug content in normal tissues returned to near background levels. The variety of iodine isotopes available makes iodothiouracil a particularly promising melanoma-localizing agent. Tumor uptake and biodistribution of [^{35}S]thiouracil and iodothiouracil (both iodine-127 (^{127}I) and iodine-125 (^{125}I) labeled) have been compared and were found to be essentially the same. The selectivity of [^{125}I]thiouracil for melanoma has been qualitatively demonstrated by autoradiography of whole-body sections and quantitated by analysis of tumor and selected tissues. Iodothiouracil was also shown to localize in remote secondary metastases using a metastatic variant of the Harding-Passey melanoma currently being developed in our laboratory. These studies confirm the melanoma localizing capabilities of an iodinated thiouracil, and therefore the potential of using iodinated thiouracil derivatives for diagnosis and therapy of melanotic melanomas.

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Malignant melanoma is one of the most lethal of cancers. Although deaths attributable to melanomas comprise only ~1% of all cancer deaths, the high percentage of melanomas that metastasize, the difficulty in locating secondary metastases, and the refractoriness of these widespread metastases to treatment all tend to make the long-term survival outlook for patients with malignant melanoma poor. It has been estimated that the 5-year survival rate is as low as 14% (*1*). An exception to this scenario is cutaneous melanoma that has been diagnosed and excised before the lesion exceeds a critical thickness. When cutaneous malignant melanoma is removed before reaching a thickness of 0.76 mm the survival rate is 95–100% (*2,3*). Once the lesion exceeds this thickness, however, the probability of metastasis is greatly increased (*4*). There is at present no effective treatment for metastatic melanoma.

The systemic administration of nonselective cytotoxic agents as a means of tumor therapy has been limited by deleterious effects resulting from uptake into rapidly proliferating normal tissues such as bone marrow and the intestinal epithelium. A unique and potentially exploitable characteristic of melanomas is the presence of the pigment melanin within the tumor cells. A number of compounds have shown affinity for the preformed melanin pigment, of which chlorpromazine is one of the most closely studied (*5*). Systemic application of these compounds, however, also results in accumulation of these agents in normal tissues which also contain the melanin pigment such as eye and skin. In the case of ocular melanoma the choroid of the eye may contain more melanin than choroidal melanoma. The uptake of melanin-affinic agents in background tissues with high melanin content has precluded the successful use of these agents in the detection of melanomas or as vehicles for the delivery of therapeutic amounts of radionuclides to the tumor site (*6*).

Thiouracil has been shown to be selectively incorporated into the melanin pigment of melanomas by serving as a false melanin precursor (*7–11*). Whittaker has proposed that thiouracil condenses with quinone intermediates in the melanin biosynthetic pathway and is incorporated into the melanin polymer by way of a thiouracil-quinone bond that is highly resistant to degradation (*12*). The fact that thiouracil is incorporated only during melanogenesis and shows no affinity for

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preformed melanin (*7–10,12*) circumvents the background problems inherent in other melanin-affinic agents. Various derivatives of thiouracil have been shown to accumulate in melanomas (*13–15*). The 5-iodo derivative of thiouracil is of particular interest to us. When labeled with the appropriate isotope of iodine, for example iodine-123 (^{123}I), which has suitable characteristics for imaging, iodothiouracil holds great potential for use as a diagnostic agent in the imaging of both primary melanomas and remote secondary metastases using planar as well as single photon emission computed tomography (SPECT). Iodothiouracil should also serve as a vehicle for delivering therapeutic doses of radiation directly to the tumor when labeled with iodine-131.

The results presented here extend our previous work with [^{35}S]thiouracil (*9*) and demonstrate that iodothiouracil is incorporated into melanomas to the same extent as [^{35}S]thiouracil with similar tumor:normal tissue ratios. We have been able to obtain tumor uptake of iodothiouracil that is significantly higher than that reported by others (*13–15*) and should be adequate for both diagnosis and therapy. A neutron activation analysis technique has been developed for the detection of ^{127}I in biologic samples which enables us to utilize [^{127}I] thiouracil for uptake and biodistribution experiments when it is not possible or not desirable to use radiolabeled thiouracil. We have synthesized iodine labeled thiouracil using both stable ^{127}I as well as ^{125}I in order to establish the techniques necessary for the production of thiouracil labeled with ^{123}I and ^{131}I. Finally, preliminary data are presented on [^{127}I]thiouracil incorporation into remote secondary metastases using a metastatic melanoma cell line currently being developed and characterized in our laboratory.

MATERIALS AND METHODS

The sodium salt of 5-iodo-2-thiouracil was purchased commercially. Sulfur-35-labeled thiouracil (specific activity 100–300 mCi/mmol) and carrier-free Na ^{125}I (~1,700 Ci/mmol) were purchased commercially.* 5-iodothiouracil was prepared from thiouracil in three steps as follows. First, the sulfur was blocked with a benzyl group as described by Wheeler and Liddle (*16*). The S-2-benzyl thiouracil was then iodinated according to the method of Johnson and Johns (*17*) for the preparation of 5-iodo-2-ethylthiouracil. Finally, the benzyl protecting group was removed (*18*) to yield 5-iodothiouracil. Radioiodine-labeled thiouracil was prepared by adding ^{125}I, (supplied as Na ^{125}I in 0.1*M* NaOH) along with the carrier iodine during the iodination reaction (*17*). The nonradioactive 5-iodothiouracil produced by this method was identical to the authentic material as judged by the following criteria. The observed melting point was 228–232°C (dec); the literature value (*18*) is 228–230°C (dec). When chromatographed on silica gel thin layer plates, both the synthetic and authentic 5-iodothiouracil exhibited the following mobilities: solvent chloroform/ethylacetate 1:1, Rf = 0.71; solvent chloroform/methanol 9:1, Rf = 0.59. Both the synthetic and commercial 5-iodothiouracil exhibited the following uv spectral properties: absorption maxima at 274 and 314 nm, and an absorption minimum at 294 nm. Sephadex G-10 column chromatography was also used to verify the identity and purity of the 5-iodothiouracil. Columns (0.5 cm × 7 cm) were equilibrated and eluted with 20 m*M* acetic acid; the sample volume applied to the column was 0.1 ml and 1.0 ml fractions were collected. Thiouracil eluted in fractions 3–4 whereas 5-iodothiouracil eluted from the column in fractions 12–15. Elemental analysis of the 5-iodothiouracil prepared in our laboratory showed the material to exist as the sodium salt, monohydrate.

Anal. Calcd. for $C_4H_4N_2O_2$ ISNa: N, 9.52; I, 43.16. Found: N, 9.52; I, 42.74.

The identity of the ^{125}I-labeled 5-iodothiouracil was verified by thin layer chromatography (TLC) on silica gel using the two solvent systems described above. When the TLC plates were cut into sections, the region containing the 5-iodothiouracil, as visualized by uv absorbance, contained >95% of the applied counts of ^{125}I. Further, when the [^{125}I]thiouracil was chromatographed on Sephadex G-10, as described above, 99.3% of the activity applied to the column eluted in the region corresponding to 5-iodothiouracil. Free iodide eluted from the column in fraction 3. The radiochemical yield of [^{125}I]thiouracil was 5–10% based on Na ^{125}I. The specific activity of the [^{125}I]thiouracil was 0.17 μCi per μmole.

Solutions for injection were prepared in phosphate buffered saline (2.68 m*M* KCl, 1.47 m*M* KH_2PO_4, 136.9 m*M* NaCl, 8.1 m*M* Na_2HPO_4, pH = 7.2) and sterilized by passage through a 0.22-μm filter before use. All injections were given i.p.; our previous work has shown no differences for tumor and tissue uptake following either i.p. or i.v. injection (*5,9*). Uptake experiments were carried out in adult female BALB/c mice carrying the Harding-Passey melanoma (*5*); mice weighed ~20 g and were ~10 wk old. This tumor model is maintained by subcutaneous implantation of minced tumor tissue on the abdomen. All mice used in each experiment were implanted with the same tumor stock. Uptake experiments were performed 2–3 wk following implantation when the tumor size reached 100–400 mg. We have found that, for tumors in the range of 100–400 mg, the incorporation of thiouracil is not a function of tumor size. Tumors above and below this size range were not used due to the greater variability in thiouracil uptake. Mice were killed under ether anes-

thesia, dissected, and the individual tissue and organ samples were analyzed for thiouracil content as described below. Uptake values are expressed as percent of the injected dose per gram of freshly dissected tissue.

Tissue samples from [^{35}S]thiouracil experiments were prepared for liquid scintillation counting as previously described (*6*). Tissue samples containing [^{125}I]thiouracil were counted in a Nuclear Chicago 1185 Series well-type Automatic Gamma Counting System. The distribution of [^{127}I]thiouracil was determined using a neutron activation analysis technique. Freshly dissected tissue samples ranging from 0.1–0.5 g were placed in 2-ml polypropylene cryogenic storage vials and the volume was adjusted to 1.8 ml with distilled water. The samples were activated at the tangential pneumatic irradiation tube of the Medical Research Reactor at Brookhaven National Laboratory by exposure to a neutron flux density of 7.2×10^{12} neutrons/cm^2 sec at 2 MW for 5 min. The neutron bombardment converts ^{127}I to the unstable isotope ^{128}I which decays ($t_{1/2}$ = 25 min) to xenon-128 emitting a 443 keV gamma-ray in the decay process. The gamma emission from the activated tissue samples was resolved using a 2 × 2 in. pure germanium crystal detector† and quantitated by use of a multichannel analyzer.‡ Each sample was counted for two separate 200-sec periods and the net counts above background were corrected for the decay that had occurred since the end of the neutron activation. Appropriate corrections were made for the background gamma emission of irradiated tissue samples that were not treated with iodinated thiouracil by subtracting background counts from an area outside the region of interest proportional to the area under the ^{128}I signal. The concentration of a stock solution of 5-iodo-2′-deoxyuridine was detemined by measuring the uv absorbance (absorption maximum, 288 nm; molar extinction coefficient, 7413) and aliquots of this stock solution served as iodine standards for the neutron activation analysis. The reproducibility of the neutron activation analysis method has been found to be plus or minus 5% (s.d.) on replicate analyses of the standard iodine solution (n = 6). The background emission due to irradiated tissue is proportional to the size of the tissue sample. The ratio of background compared with signal will vary depending on the relative amounts of iodine and tissue present in the activated sample. For a 200-mg tissue sample containing 1 μg I the signal-to-noise ratio is ~2. The sensitivity of this method is not as great as that using [^{125}I]thiouracil, especially if high specific activity ^{125}I can be used. We estimate the limit of detection of ^{127}I by the neutron activation method to be 0.5 ppm.

Whole-body autoradiographs were prepared according to the technique developed by Fand and McNally (*19*) from 3-μm sections of BALB/c mice killed 24 hr after i.p. injection of [^{125}I]thiouracil.

RESULTS

Table 1 shows the uptake of [^{35}S]thiouracil in various organs of mice bearing Harding-Passey melanoma. Accumulation in melanoma was substantially greater than any of the other tissues investigated. These data are consistent with those we have reported previously (*9*) but were retaken for these experiments to ensure a valid comparison between [^{35}S]thiouracil and I-thiouracil. After the tumor, the liver was the organ that showed the highest uptake of [^{35}S]thiouracil. Higher tumor to normal tissue concentration ratios were observed when tissues were analyzed at 24 hr postinjection compared with 4 hr, reflecting the greater length of the time allowed for clearance of the drug from normal body tissues. It should be noted that the tumor and tissue levels of [^{35}S]thiouracil are similar regardless of whether a single injection or a series of five injections are given. This indicates that, at least for the trace doses administered here, the mechanisms of normal tissue clearance and tumor binding are not being saturated.

Initial measurements of [^{127}I]thiouracil biodistribution were carried out using commercially obtained material. Figure 1 shows that the tumor and organ uptake of [^{127}I]thiouracil is similar to that observed with carbon-14 thiouracil (*9*) and [^{35}S]thiouracil (Table 1). Table 2 shows the results of tumor and organ uptake studies carried out with [^{127}I]thiouracil and [^{125}I]thiouracil synthesized in our laboratory (radiolabeled iodothiouracil is not available commercially). These results compare reasonably well with the uptake observed with the commercial [^{127}I]thiouracil (Fig. 1) and [^{35}S]thiouracil (Table 1). The variability of the tumor/blood ratio values presented in Table 2 are due in part to experimental error involved in measuring very small levels of [^{125}I]thiouracil, particularly at the 48-hr time point.

TABLE 1
Tumor and Organ Uptake of [^{35}S]Thiouracil in BALB/c Mice Carrying Harding-Passey Melanoma (% Dose/g Tissue)*

Tissue	Single dose†	Single dose‡	Multiple dose§
Melanoma	7.11(4.84–10.37)	8.52(8.02–9.03)	6.91(6.08–7.51)
Blood	0.23(0.21–0.27)	0.06(0.05–0.08)	0.42(0.21–0.63)
Liver	1.38(1.24–1.65)	0.73(0.67–0.78)	1.29(1.02–1.63)
Lung	0.60(0.56–0.64)	0.19(0.17–0.21)	0.58(0.36–0.75)
Kidney	0.39(0.35–0.45)	0.14(0.12–0.16)	0.58(0.33–0.82)
Spleen	0.19(0.17–0.25)	0.09(0.08–0.10)	0.32(0.20–0.41)
Muscle	0.12(0.11–0.15)	0.05(0.04–0.06)	0.20(0.14–0.27)
Brain	0.10(0.09–0.11)	0.04(0.03–0.05)	0.17(0.09–0.23)

* Values are mean, numbers in parentheses are range.

† Single i.p. injection of 0.10 μCi [^{35}S]thiouracil; mice killed 4 hr postinjection (n = 3).

‡ Single i.p. injection of 0.10 μCi [^{35}S]thiouracil; mice killed 24 hr postinjection (n = 2).

§ Five i.p. injections of 0.10 μCi [^{35}S]thiouracil each, total dose 0.5 μCi, one injection every 6 hr for 24 hr; mice killed 4 hr after final injection (n = 3).

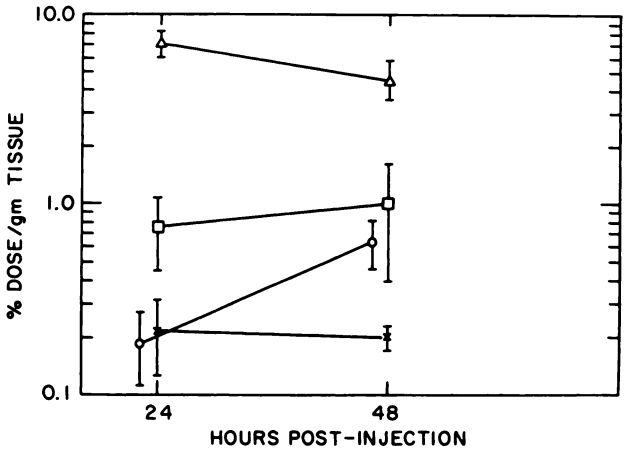

FIGURE 1
Tumor and organ distribution of commercially obtained [^{127}I]thiouracil in BALB/c mice carrying Harding-Passey melanoma. Each mouse received single i.p. injection (0.2 ml) of 440 μg [^{127}I]thiouracil and was killed at indicated times. Each point is average of three mice, bars represent s.d. Tissue samples were analyzed for ^{127}I by neutron activation analysis. (△) Tumor; (□) Blood; (X) Muscle; (○) Liver

Values for [^{127}I]thiouracil levels in tissue were obtained by neutron activation analysis. These measurements are subject to additional statistical uncertainty due to the subtraction of a large background from a small signal.

The data presented in Table 2 results from the injection of relatively large amounts of I-thiouracil; 405 μg of [^{127}I]thiouracil and 2.4 mg of [^{125}I]thiouracil. Even following the injection of such a large amount of material, the clearance of I-thiouracil from normal body tissues is still effective as evidenced by the very low values obtained for [^{125}I]thiouracil at 48 hr postinjection. It is possible that the large amounts of carrier [^{127}I] thiouracil have decreased the amount of drug incorporated into tumor (see Table 1).

To determine the extent of deiodination of [^{125}I] thiouracil, a control experiment was carried out in which one group of mice received 0.2% KI in their drinking water to saturate the iodide binding sites in the thyroid. The thyroid uptake values with or without KI blocking were found to be the same (Table 2), indicating that deiodination of [^{125}I]thiouracil was not taking place to any appreciable extent during the course of this experiment.

Figure 2 shows whole-body counts of three tumor-bearing BALB/c mice that had each received a single intraperitoneal injection of 1 μCi [^{125}I]thiouracil. The biologic half-life of nontumor-bound [^{125}I]thiouracil is observed to be ~2 hr, the majority of the injected radioactivity being excreted in the urine. The whole-body counts decrease to roughly 10% of the original value and begin to level off. This residual level represents the [^{125}I]thiouracil bound to the melanin in the tumor.

We have investigated the long-term persistence of [^{35}S]thiouracil in tumor and normal tissues. Figure 3 shows data from an experiment where mice were injected with 2.6 μCi of [^{35}S]thiouracil and the residual levels of [^{35}S]thiouracil determined at times up to 2 wk. Data was also obtained from the following tissues not shown in Fig. 3: lung, gut, spleen, and pancreas. The values for these organs were all intermediate between those shown for kidney and blood and have been omitted from Fig. 3 for clarity. The dashed line in Fig. 3

TABLE 2
Tumor and Organ Uptake of [^{125}I]Thiouracil and [^{127}I]Thiouracil in BALB/c Mice Carrying Harding-Passey Melanoma (% Dose/g Tissue)*

Tissue	[^{125}I]TU 24 hr	[^{125}I]TU + KI 24 hr‡	[^{127}I]TU 24 hr	[^{125}I]TU 48 hr	[^{127}I]TU 48 hr
Melanoma	3.6 ± 0.69	4.16 ± 1.04	5.45 ± 2.52	2.70 ± 0.84	6.22 ± 1.98
Blood	0.17 ± 0.01	0.21 ± 0.38	0.81 ± 0.16	0.040 ± 0.005	1.4 ± 0.7
Liver	0.16 ± 0.008	0.16 ± 0.15	0.78 ± 0.37	0.075 ± 0.006	0.67 ± 0.14
Lung	0.10 ± 0.007	0.129 ± 0.025		0.026 ± 0.004	
Kidney	0.10 ± 0.007	0.134 ± 0.036		0.038 ± 0.009	
Spleen	0.02 ± 0.007	0.032 ± 0.005		0.009 ± 0.003	
Muscle	0.01 ± 0.001	0.018 ± 0.005	0.41 ± 0.07	0.002 ± 0.001	0.63 ± 0.40
Gut	0.04 ± 0.005	0.047 ± 0.011		0.005 ± 0.001	
Brain	0.005 ± 0.001	0.007 ± 0.002		0.001 ± 0.0003	
Thyroid†	0.026 ± 0.009	0.021 ± 0.008		0.016 ± 0.003	

*Both [^{125}I]thiouracil and [^{127}I]thiouracil were prepared in our laboratory. Each mouse received 1.4 μCi (2.4 mg) [^{125}I]thiouracil or 405 μg [^{127}I]thiouracil via i.p. injection and was killed at times indicated; each time point is average of four mice for [^{125}I]thiouracil and three mice for [^{127}I]thiouracil. Values are mean ± s.d.

† % injected dose per organ.

‡ 0.2% KI in drinking water for 4 days prior to injection.

represents the amount of [^{35}S]thiouracil incorporated per gram of tumor after correction for the dilution due to tumor growth. The observed tumor levels were multiplied by a factor corresponding to the increase in tumor volume from the start of the experiment. The experimentally observed average tumor doubling time of 3 days was used for this correction. As has been previously reported (*12*), these results indicate that the thiouracil-melanin bond is extremely stable and that thiouracil bound to melanin can be considered as permanently incorporated.

Table 3 shows the uptake and distribution of [^{127}I] thiouracil in BALB/c mice carrying a Harding-Passey melanoma which has been selected in our laboratory for the ability to metastasize from the original subcutaneous implantation site to other organs and lymph nodes. This metastatic melanoma model will be described in detail elsewhere. These data demonstrate that iodothiouracil is taken up equally well in secondary metastases as in the primary melanoma.

An autoradiograph displaying multiple remote metastatic sites in a BALB/c mouse is shown in Fig. 4. This picture was obtained from an animal killed 24 hr after the last of a series of i.p. injections (five injections over 24 hr; one every 6 hr). The total dose injected was 7.5 μCi of [^{125}I]thiouracil. The areas of radioactivity in the abdominal cavity correlate with verified sites of tumor metastasis. In 25 such pairs of sections and autoradiographs from each of two mice, all areas of exposed film correlated with tumor, qualitatively demonstrating the specificity of [^{125}I]thiouracil for melanoma.

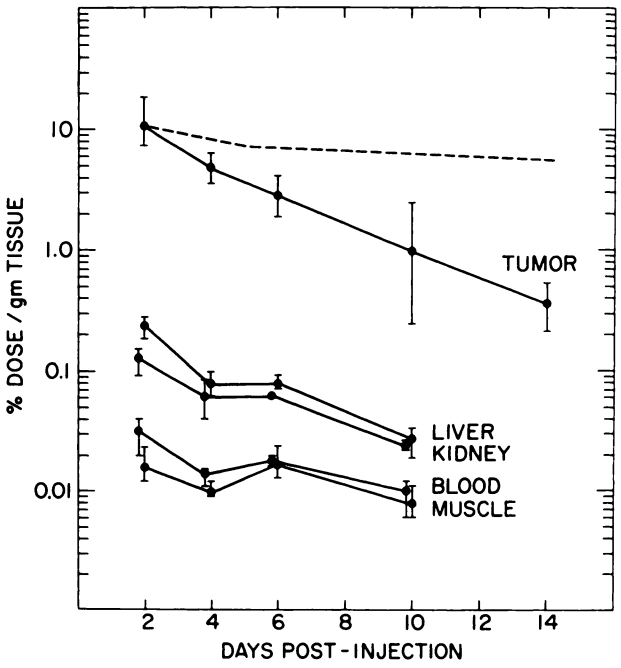

FIGURE 3
Long-term persistence of [^{35}S]thiouracil in tumor and normal tissue. All mice received single i.p. injection (0.2 ml) of 2.6 μCi [^{35}S]thiouracil. Each point is average of three mice, bars indicate range. Dashed line represents amount of [^{35}S]thiouracil incorporated per gram of tumor after correcting for dilution due to tumor growth by multiplying measured value by factor corresponding to increase in tumor volume.

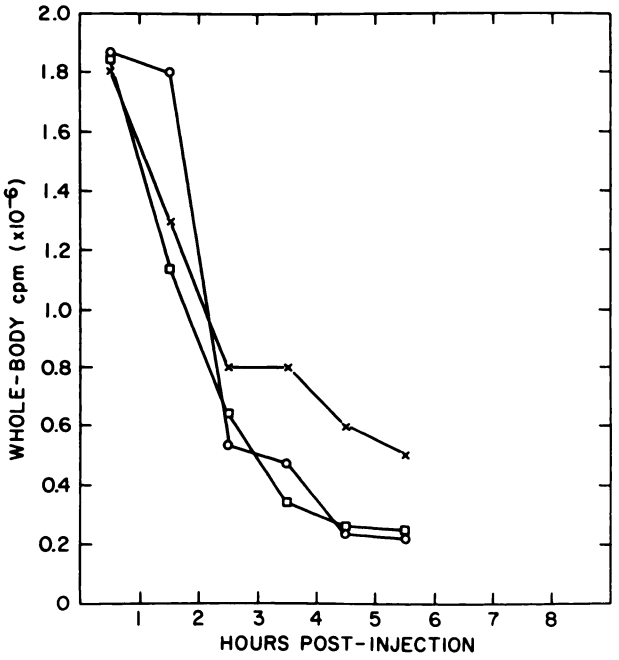

FIGURE 2
Whole-body clearance of [^{125}I]thiouracil following single i.p. injection. Three BALB/c mice carrying Harding-Passey melanoma were each injected with 1 μCi of [^{125}I]thiouracil in volume of 0.2 ml. Each mouse was then counted at indicated times in horizontal NaI well-type gamma detector to determine residual whole-body levels of [^{125}I]thiouracil. Observed biologic half-life of [^{125}I]thiouracil is ~2 hr

TABLE 3
Tumor and Organ Uptake of [^{127}I]Thiouracil in Metastatic Harding-Passey Melanoma Model in BALB/c Mice*

Tissue	[^{127}I]thiouracil (% dose/g tissue)	
Blood	1.4 ± 0.7	n = 3
Liver	0.67 ± 0.14	n = 3
Muscle	0.63 ± 0.4	n = 3
Melanoma		
Subcutaneous implantation site:	5.7 ± 2.1	n = 4
Intra-peritoneal invasion site	7.1 ± 1.4	n = 5
Liver metastasis	3.5 ± 0.2	n = 2
Mesenteric lymph node metastases	7.2 ± 1.2	n = 2

* Single i.p. dose (405 μg [^{127}I]thiouracil), 48 hr postinjection. Values are mean ± s.d.

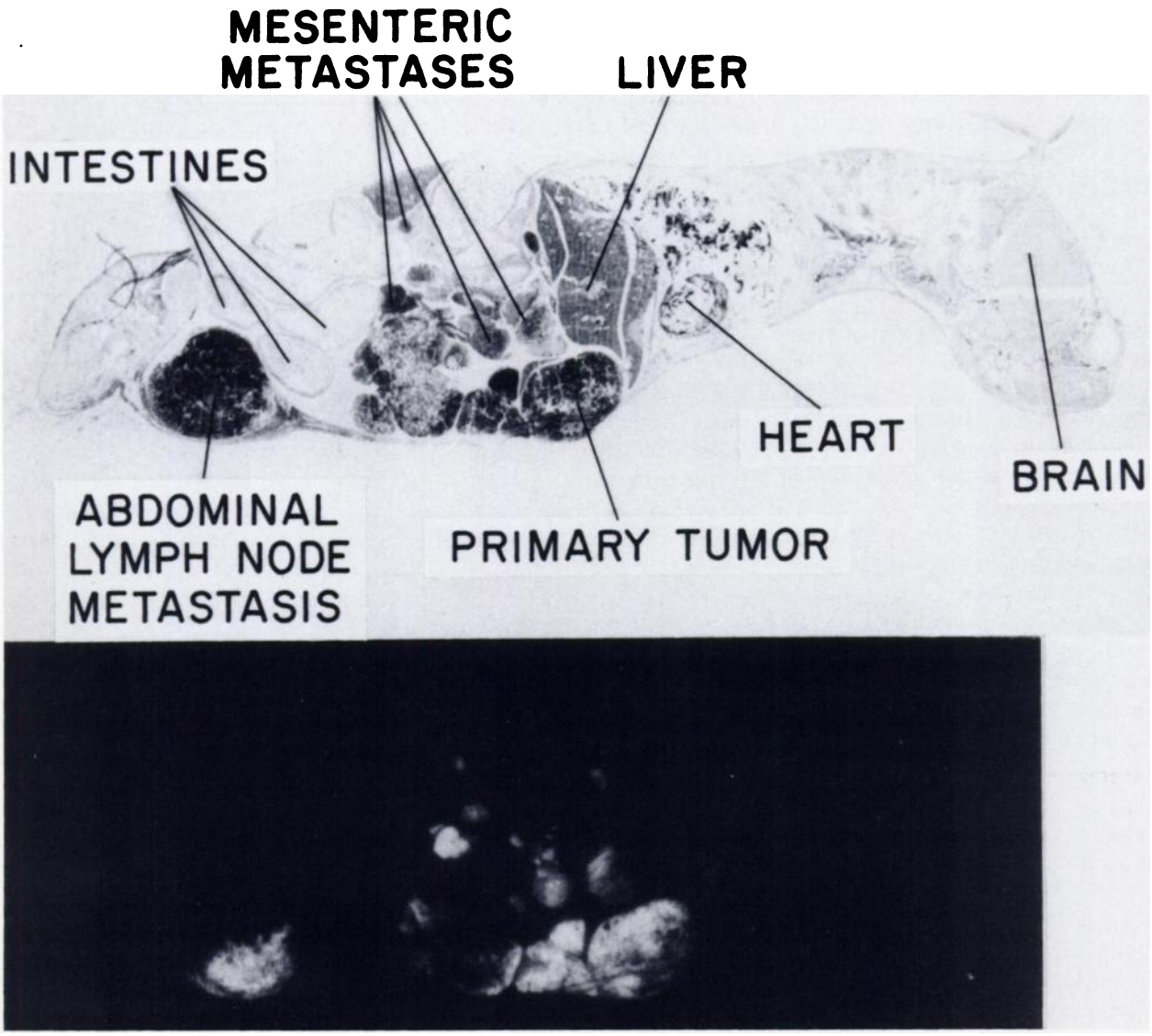

FIGURE 4
Whole-body autoradiograph of [^{125}I]thiouracil in BALB/c mouse carrying metastatic Harding-Passey melanoma. Total of 7.5 μCi of [^{125}I]thiouracil was injected (5 i.p. injections over 24 hr); mouse was killed 24 hr after final injection. Series of 25 sections and corresponding autoradiographs was prepared. In all sections areas of exposed film correlated with verified tumors. Section presented here clearly shows both primary tumor and remote metastatic sites. Exposure time for autoradiograph was 30 days

DISCUSSION

The selectivity of thiouracil and various thiouracil derivatives for growing melanin makes these compounds ideal for the detection and treatment of melanomas. Once incorporated into the melanin pigment, the thiouracil remains tightly bound while the thiouracil distributed in normal tissues is cleared. Whole-body gamma counting of tumor bearing mice following an i.p. injection of [^{125}I]thiouracil indicated that the biologic half-life of the nontumor-bound compound is ~2 hr. This selectivity of thiouracil uptake by growing melanomas and the rapid clearance of nonbound thiouracil results in tumor/background tissue ratios that are suitable for scintigraphy as well as for potential therapeutic applications.

The results described above extend our previous results with [^{35}S]thiouracil (*9*) and demonstrate that iodothiouracil is incorporated into tumors to about the same extent as thiouracil. This is in agreement with the work of others (*13–15*), although in our studies, tumor uptake (of both [^{35}S]thiouracil and iodothiouracil) is significantly greater than values reported by others. Using the Harding-Passey melanoma model in BALB/c mice we have consistently observed tumor uptake values which range from 5–12% of the injected dose per gram tumor. Values reported in the literature (*13–*

15) for the incorporation of iodothiouracil into melanomas are 10–100-fold lower than the values which we obtain. Van Langevelde et al. (*14*) use the Green melanoma in Syrian golden hamsters and reported a tumor incorporation of only 0.14% of the dose/g tissue 24 hr after injection of [^{131}I]thiouracil. Perhaps more important, however, is the fact that at 24 hr postinjection the levels of [^{131}I]thiouracil in liver and kidney exceeded that observed in tumor while the tumor/blood ratio was only 1.75. The Greene melanoma as carried in our laboratory has a melanin content that is one-half the content of Harding-Passey melanoma (*6*); this would explain the discrepancy only in part. Larsson et al. (*13*, *15*) on the other hand, used the Harding-Passey melanoma model in DBA mice for uptake studies and still obtained tumor incorporation values which are 5–10-fold lower than we have been able to demonstrate. While a possible lower melanin content in the tumor models used in previous studies (*13–15*) may contribute in part to the observed discrepancies, the extent of this effect is impossible to determine as melanin contents were not provided in these reports.

We have undertaken a fairly extensive analysis of melanin content in various human tissues (*6*), and found that the value for well-pigmented human melanomas varied from 0.1 to 0.9% melanin by weight (average value = 0.35%). This compares well with the value of 0.68% by weight for our Harding-Passey melanoma, and this verifies that the melanin content of our model is within the range found in human melanomas. In view of the direct relationships between melanin content of melanomas and uptake of melanin-affinic compounds (*5*) it would appear mandatory to carefully ascertain and report melanin content of any tumor models used to evaluate potential melanoma-seeking agents for either diagnosis or therapy.

Another possible reason for lower uptake values reported by others is the deterioration of biological activity of radiolabeled thiouracil following prolonged storage in solution. We have shown that storage of [^{14}C] thiouracil at 5°C in 0.1*M* Tris buffer at pH 8 for 4 mo resulted in a 2.5-fold decrease in the apparent uptake in tissue compared to freshly prepared solutions (*9*). For all of our experiments, solutions of radiolabeled thiouracil are freshly prepared from the stored solid. When storage is necessary the solutions are frozen, and checked by TLC before use to ensure that the compound has not decomposed.

Iodothiouracil is of particular interest to us because of the variety of iodine isotopes available. Iodine-123, a short-lived gamma emitter should be useful for imaging small primary tumors and for the detection of remote metastatic sites. With ^{131}I-labeled thiouracil it should be possible to deliver therapeutic doses of radiation directly to the tumor while minimizing damage to critical target organs such as bone marrow and intestinal epithelium. Based on measurements of the amount of thiouracil bound to gut (see Table 2) and bone marrow (*9*) the dose delivered to these organs should be only 2 and 5%, respectively, compared with that received by the tumor. For therapy experiments [^{131}I]thiouracil may be the compound of choice due to the higher energy and significantly greater penetration of the beta$^-$ radiation. Iodine-131 thiouracil may be preferred over [^{35}S] thiouracil because the 106 keV beta$^-$ emitted by ^{131}I has an average range of ~70 cell diameters compared to approximately ten cell diameters for ^{35}S (*20*).

The usefulness of iodinated thiouracil for diagnosis of melanotic melanoma (imaging) depends more on the ability to achieve high tumor:normal tissue ratios of drug incorporation rather than on the absolute amount accumulated in the tumor. In the experiments reported here, we have been able to demonstrate consistently high tumor:normal tissue levels of both [^{35}S]thiouracil and iodothiouracil. These ratios should be sufficient to minimize damage to other organs during therapy experiments and to minimize background interference during imaging procedures.

We are in the process of developing and characterizing a metastatic variant of the Harding-Passey melanoma. In the BALB/c mouse this tumor closely mimics the behavior of the human melanoma, metastasizing widely from an initial implantation site, and retaining its pigmentation in the secondary sites. Human melanomas metastasize to a wide variety of tissues; in particular, choroidal melanoma, in 85% of cases metastasizes to the liver. If use of the appropriately labeled iodothiouracil enables these metastases to be dectected at an early state of development, the chances of successful therapy should be greatly enhanced. Our results show that iodothiouracil is taken up equally well in the remote metastatic sites as in the primary tumor.

Iodinated thiouracil, therefore, seems to be a most promising drug for the localization and treatment of malignant melanomas. We have synthesized [^{125}I]thiouracil and shown that the tumor uptake and body distribution is virtually identical to that of thiouracil. The iodination procedure is suitable for incorporation of both ^{123}I and ^{131}I. Iodine-123-labeled thiouracil will be potentially a very useful agent for detection and localization of both primary and metastatic melanoma using planar imaging as well as SPECT. Likewise, ^{131}I-labeled thiouracil shows promise as a radiotherapeutic agent for melanoma. Studies are in progress to determine the feasibility of these procedures.

FOOTNOTES

* Amersham Corporation, Arlington Heights, IL.

† Ortec, Oak Ridge, TN.

‡ Tracor Northern TN 1705, Tektronx Inc., Beaverton, OR.

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