Non-utilization of Radioactive Iodinated Uracil, Uridine, and Orotic Acid by Animal Tissues in Vivo*

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Adenine (1, 6), guanine (1, 8, 5), cytidine (13), deoxyctydine (18), thymidine (18), and orotic acid (2), can be utilized by certain mammalian organisms for the synthesis of nucleic acids; and the rate of incorporation of many of these compounds into the nucleic acids of rapidly growing tissues, such as regenerating liver or neoplastic tissues, is greater than into those of resting tissues (10, 21).

Although 8-azaguanine is not a naturally occurring compound, evidence that it can be incorporated to a small extent into mammalian nucleic acids has been presented (16). This analog of guanine markedly inhibited the growth of Tetrahymena geleii, a guanine-requiring protozoan, and of certain experimental tumors. Kidder et al. (14) suggested that the carcinostatic properties of the analog might be explained on the basis of a requirement for guanine for the synthesis of nucleic acid by cancer cells. However, Mitchell and his co-workers (16) and Bennett et al. (4) were unable to demonstrate preferential utilization for nucleic acid synthesis of 8-azaguanine-2-C14 by a susceptible tumor (E 0771).

In view of the observations regarding the incorporation of 8-azaguanine into nucleic acids and the fact that there is a much greater rate of nucleic acid synthesis in tumor tissue than in the corresponding normal tissue of the same animal, this study was undertaken to investigate the possibility of a preferential concentration by tumor tissue of I131-derivatives of naturally occurring pyrimidines. An incorporation of I131-labeled pyrimidines could be of diagnostic and possibly of chemotherapeutic value. Considerable success has been attained in the use of I131-labeled diiodofluorescein derivatives (17), iodinated human serum albumin (8), and sodium iodide (9) in the isotope-encephalographic localization of brain tumors. Furthermore, an I131-labeled oxazine dye had a significant effect in prolonging the life of mice bearing transplanted tumors (19). If an effective and easily synthesized radioactive iodine-labeled compound could be found, the possibility might be afforded of the comparable use of compounds labeled with eka-iodine (astatine211), a potent emitter of alpha particles, although this element is prepared with difficulty and has the inconveniently short half-life of 7.5 hours (18).

Three I131-labeled pyrimidines, iodouridine-5-I131, iodouracil-5-I131, and iodoorotic acid-5-I131, were synthesized, and their incorporation into normal tissue, regenerating liver, and certain experimental tumors was investigated. The results revealed that none of these compounds is incorporated to any significant degree into any of the tissues studied.

EXPERIMENTAL

Synthesis of iodoorotic acid-5-I131.—To a solution of radioactive iodide (5 mc.; 1 mμg.; 0.8 ml.), contained in a 15 ml.-centrifuge tube, was added NaOH (10 per cent, 0.1 ml.) and carrier KI (4 mg.). The centrifuge tube was fitted with a stopper containing inlet and outlet tubes, and the solution was then concentrated to a volume of 0.2 ml. by passing a jet of dry air over it while it was heated on the steam bath. The exhaust air was led through a cold carbon tetrachloride trap and finally directly into the flue of the fume hood.

The concentrated solution was cooled in an ice bath, and the iodide was oxidized by the addition of H2SO4 (18 N, 0.2 ml.) and 0.005 M KIO3 (0.3 ml.). A solution of orotic acid (200 mg.) in NaOH (3 N; 2 ml.) was added, and this was followed immediately by iodine (320 mg.). The reaction mixture was heated on the steam bath for 15–20 minutes. During the heating, a further addition of NaOH (3 N) was made if necessary to effect complete solution of the iodine. The reaction mixture was cooled in ice; upon acidification with acetic acid a white

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centrifuged off, and the supernate was discarded. In an attempt to remove any unreacted iodine, the precipitate was washed with 1:1 ethanol-acetic acid until the supernate was colorless, then once with ethanol and twice with ether. The iodoorotic acid was then recrystallized twice from 50 per cent ethanol.

With rapid heating the free acid decomposed at 270°-278° C. with the evolution of iodine vapors. In several syntheses the incorporation of radioactive iodine ranged from 40 to 50 per cent of the theoretical value. The elementary analysis was: found: C, 21.37; H, 0.97; N, 10.14; I, 44.89; calculated: C, 21.26; H, 1.06; N, 9.94; I, 45.03. When the radioactive iodoorotic acid was chromatographed, using the iso-amyl alcohol-5 per cent Na2HPO4 system of Carter (7), the center of radioactivity, measured in a well-type scintillation counter, coincided with the ultraviolet absorbing spot Rf, 0.89, as located by a Mineralite ultraviolet lamp, model SL-2537. In NaOH (0.01 n), the ultraviolet absorption spectrum showed a maximum at 303 mμ (ε = 9,170) and a minimum at 255 mμ; and in HCl (0.01 n) a maximum at 287 mμ (ε = 6,460) and a minimum at 246 mμ were observed.

The position of the iodine atom has been assigned to carbon-5 for the following reasons. Hydroxy (oxy) groups are already attached to the carbon atoms at the 2 and 4 positions, and a carboxyl group is attached to carbon-6 (the new numbering system of Chemical Abstracts is used). If the iodine together with the carboxyl group was attached to the carbon at position-6, the typical ultraviolet absorption spectrum characteristic of a pyrimidine structure would have been destroyed because of inhibition of resonance caused by saturation of the 5-6 double bond. For a similar reason, the iodine together with a hydroxy group could not be attached to the carbon at either position-2 or -4. The possibility of substitution of either of the hydroxy groups at positions 2 and 4 is ruled out by the elementary analysis. The formation of a hydrogen iodide complex by orotic acid is not only highly improbable, because of the absence of any basic groups in the molecule, but, in addition, is also ruled out by the fact that the iodoorotic acid retained the theoretical amount of iodine after four recrystallizations from HCl (0.1 n). Therefore, the only available position left for substitution by iodine, which satisfies the spectral data and the theoretical amount of iodine after four recrystallizations from HCl (0.1 n), is position 5 of the pyrimidine ring.

Synthesis of iodooridine-131.—Radioactive iodide solution (10 mc.), as obtained from Oak Ridge, and a solution of KI (0.06 M, 0.3 ml.) were pipetted into a 25-ml. round-bottomed flask (glass-stoppered) and evaporated to 0.5 ml. by heating on a steam bath while passing a stream of air into the flask. After the addition of HNO3 (8 n, 0.5 ml.) to oxidize the iodide to free iodine, water (25 ml.), uridine (200 mg.), iodine (200 mg.) and chloroform (2 ml.) were added, and the resultant mixture was refluxed on a steam bath for 8 hours. All operations were performed in the hood, and special precautions were taken to minimize contamination of the atmosphere with radioactive iodine.

All joints were sealed with silicone, and air was blown through the top of the condenser to carry any iodine vapors into a CHCl3-trap which was cooled in an ice bath; emitted air was passed directly into the flue of a fume hood. During refluxing, clusters of long white needles of iodooridine were formed. After cooling overnight at 5° C., the chloroform and aqueous layers were discarded, and the remaining crystals of iodooridine were washed with ether until the solvent was colorless. The yield at this point was 200 mg., with 32 per cent of the original I131 incorporated or 64 per cent of theory. The compound was further purified by treatment with Nuchar (C250) and by recrystallization from hot water.

With rapid heating the compound decomposed at 205°-206° C. with the evolution of iodine vapors. The point of decomposition varied with the rate of heating. In NaOH (0.01 n) the ultraviolet absorption spectrum showed a maximum at 278 mμ and a minimum at 253 mμ, and in HCl (0.01 n) a maximum at 289 mμ and a minimum at 249 mμ were observed. The elementary analysis was: found: C, 29.17, 29.14; H, 2.95, 3.03; N, 7.48, 7.53; I, 34.09, 34.18; calculated: C, 29.10; H, 3.24; N, 7.50; I, 34.33.

Further evidence that this compound is 5-iodouridine was given by its reaction to bromine water; whereas addition of the latter to an aqueous solution of uridine resulted in immediate decolorization of the bromine water, one drop of this reagent retained its color when added to an aqueous solution of iodooridine. Hydrolysis of iodooridine with HClO4 (12 n) in a boiling water bath for 1 hour resulted in a compound having an Rf, similar to that of uracil in two solvent systems (7, 20), as indicated in Table 1. Violet fumes, indicating a cleavage of the organic iodine, were formed during the hydrolysis. Silver nitrate added to the acidified (HNO3) aqueous solution of iodooridine gave no

1 The elementary analyses were performed by Dr. E. W. D. Huffman, Denver, Colo.
precipitate, an indication that the iodine atom was organically bound.

*Synthesis of iodouracil-I\(^{131}\).*—The method used was a modification of that of Johnson and Johns (11). Radioactive iodide solution (5 mc.) was pipetted into a 50-ml. centrifuge tube and placed in an ice bath. A solution of KI (0.06 M, 0.1 ml.) was added, and then H\(_2\)SO\(_4\) (18 N, 0.05 ml.) and a solution of KIO\(_3\) (0.005 M, 0.3 ml.) in order to oxidize the iodide to free iodine. After the addition of a solution of uracil (200 mg.) in NaOH (2.5 N, 1.4 ml.), an I\(_2\)-KI solution was added until a definite excess of free iodine was present. Upon warming and adding additional I\(_2\)-KI solution to maintain an excess of iodine, iodouracil began to crystallize. The suspension was cooled in an ice bath for 1 hour, and then the crystallized iodouracil was removed by centrifugation. The residue was suspended in water, acidified with acetic acid (9 N), and again centrifuged. The residue was washed with acidified alcohol and then 5 times with boiling ether. The compound was recrystallized from water and then from alcohol. The yield was 276 mg. of iodouracil with 92 per cent incorporation of the original I\(^{131}\) added or 64 per cent of the theoretical value.

*Determination of radioactivity.*—The concentration of I\(^{131}\) in the tissues studied was determined with the aid of a scintillation counter constructed by MacIntyre (15). Since I\(^{131}\) emits highly penetrating gamma radiations, the tissue concentration of I\(^{131}\) could be determined directly without fractionation or isolation. In the earlier studies each tissue was dissolved in NaOH (6 N) to give a final volume of 5 ml. in a calibrated test tube. With a specially constructed test tube holder, to maintain constant geometrical relationships, the radioactivity was determined by comparing it to the same volume of a radioactive iodide solution of known specific activity. In order to eliminate the necessity of dissolving the tissue and also to improve the geometric advantage, tissues were examined subsequently by placing individual samples in a planchet and locating this directly beneath the crystal or the scintillation counter.

**RESULTS AND DISCUSSION**

The results obtained from injections of iodoactic acid, iodoacidine, and iodauracil labeled with iodine-131 into various animal species, normal and otherwise, as indicated, are shown in Table 2. No preferential incorporation of these iodinated pyrimidine derivatives into regenerating liver or experimental tumors was observed; the tumors employed were Sarcoma 180, Brown-Pearce carcinoma, adenocarcinoma E 0755, 4-dimethylaminooazobenzene-induced hepatoma, and an x-ray-induced bone tumor. Both thyroid and gastric tissues usually contained a higher concentration of radioactive iodine than did the other tissues which were investigated. Since these tissues are known to concentrate the iodide ion, the probability of the partial hydrolysis of these organic iodo-compounds in vivo is indicated. Tumors occasionally had some activity, but never more than that of other tissues. The values shown for the distribution of radioactivity among the various tissues were obtained as part of a screening program; hence, because of the limited number of animals studied, these results should not be interpreted as being a statistically acceptable tissue distribution.

**Table 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Hydrolysis</th>
<th>Hydrolysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodouriculin</td>
<td>0.52</td>
<td>0.55</td>
</tr>
<tr>
<td>Iodoacidine</td>
<td>0.68</td>
<td>0.52</td>
</tr>
<tr>
<td>Iodoacidine</td>
<td>0.70</td>
<td>0.55</td>
</tr>
<tr>
<td>Iodouriculin</td>
<td>0.60</td>
<td>0.34</td>
</tr>
<tr>
<td>Iodoacidine</td>
<td>0.71</td>
<td>0.34</td>
</tr>
</tbody>
</table>

* With 18 N HClO\(_4\) for 1 hr. in boiling water bath.

Prior to the administration of iodoactic acid, an investigation was conducted of its toxicity in white mice. Eighteen mice in groups of three each were given intraperitoneal injections of NaHCO\(_3\) (1 per cent, 0.2 ml.) containing 0, 1, 5, 10, 15, or 20 mg. of iodoactic acid. No toxic effect was observed in any of the animals.

Radioactive iodine derivatives of orotic acid, uridine, and uracil do not appear to be of diagnostic value for the localization of centers of rapid nucleic acid synthesis, and, under the conditions of these experiments, no chemotherapeutic activity was evidenced.

**SUMMARY**

Iodoacidine, iodoactic acid, and iodoacidine, labeled with I\(^{131}\), were synthesized. The syntheses of

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*2* We are grateful to Dr. Harold P. Rusch of the McArdle Memorial Laboratory of the University of Wisconsin for rats bearing 4-dimethylaminooazobenzene-induced hepatomas and mice carrying Sarcoma 180; to Dr. Alfred Gellhorn of Columbia University for donor rabbits bearing the Brown-Pearce carcinoma in the anterior chamber of the eye and donor mice bearing adenocarcinoma E 0755; and to Simon Koletsky of the Atomic Energy Research Project and the Department of Pathology of this School of Medicine for making available a rat bearing an x-ray-induced bone tumor.

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*3* We are grateful to Dr. H. L. Friedell, Dr. W. J. MacIntyre, and Mr. J. S. Krohmer for advice and assistance in the measurements of radioactivity and for supplying the I\(^{131}\), allocated to them by the Atomic Energy Commission.
### TABLE 2

**ADMINISTRATION AND LOCALIZATION OF RADIOACTIVE IODINE DERIVATIVES OF NATURALLY OCCURRING PYRIMIDINES**

<table>
<thead>
<tr>
<th>COMPOUND and SOLVENT</th>
<th>ANIMAL</th>
<th>CONDITION</th>
<th>METHOD OF ADMINISTRATION</th>
<th>TOTAL DOSE</th>
<th>TIME OF SACRIFICE</th>
<th>CONCENTRATION OF ACTIVITY</th>
<th>MICROCURIES PER GRAM WET WEIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Iodouracil-$^3$I</td>
<td>Rat (1)</td>
<td>Normal</td>
<td>IP</td>
<td>10 μc. (1.73 mg.)</td>
<td>48 hr. after injection</td>
<td>All tissues</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>Jugular</td>
<td>25 μc. (4.3 mg.)</td>
<td>24 hr. after injection</td>
<td>All tissues</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td></td>
<td>Rat (2)</td>
<td>4-Dimethylaminoazobenzene induced hepatoma</td>
<td>IV</td>
<td>60 μc. (10.5 mg.) in 3 divided doses at 24-hr. intervals</td>
<td>48 hr. after last injection</td>
<td>Thyroid</td>
<td>0.10±0.02; 0.10±0.01</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>150 μc. (25.0 mg.) in 3 divided doses at 24-hr. intervals</td>
<td>&quot;</td>
<td>Liver</td>
<td>0.25±0.13; &lt;0.005</td>
</tr>
<tr>
<td></td>
<td>Mouse (1)</td>
<td>Adenocarcinoma</td>
<td>IP</td>
<td>20 μc. (3.46 mg.) in 3 divided doses at 24-hr. intervals</td>
<td>3 hr. after last injection</td>
<td>Thyroid and trachea</td>
<td>0.92±0.14</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>5 μc. (0.86 mg.)</td>
<td>2 hr. after injection</td>
<td>Testicle</td>
<td>0.31±0.06</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>Rabbit (1)</td>
<td>Brown-Pearce carcinoma</td>
<td>IV</td>
<td>50 μc. (8.6 mg.)</td>
<td>24 hr. after injection</td>
<td>Thyroid</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>96 μc. (14.4 mg.) in 3 divided doses at 4-hr. intervals per day, for 2 days, starting 24 hr. after partial hepatectomy</td>
<td>19 hr. after last injection</td>
<td>Thyroid and trachea</td>
<td>0.20±0.02; 0.20±0.01</td>
</tr>
<tr>
<td></td>
<td>Mouse (5)</td>
<td>Sarcoma</td>
<td>IP</td>
<td>10 μc. (1.5 mg.)</td>
<td>3, 6 and 24 hr. after last injection</td>
<td>5-hr. tissues</td>
<td>0.10±0.03</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Liver</td>
<td>0.01±0.005</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Tumor</td>
<td>0.03±0.015</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Muscle</td>
<td>0.08±0.008</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Bone marrow</td>
<td>0.08±0.008</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Remainder</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

**Note:** The table includes information on the administration and localization of radioactive iodine derivatives, including the use of naturally occurring pyrimidines. The table specifies the compound and solvent used, the animal species, condition, method of administration, total dose, time of sacrifice, and concentration of activity. The concentration is given in microcuries per gram wet weight, and the table covers a range of tissues, including thyroid, stomach, liver, and others, with varying concentrations for different tissues and time periods.
iodouridine and iodoorotic acid have not been described previously. The incorporation of these three 131l-labeled pyrimidines into normal tissue, regenerating liver, and experimental tumors was investigated, and no significant preferential concentration was observed.

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Non-utilization of Radioactive Iodinated Uracil, Uridine, and Orotic Acid by Animal Tissues *in Vivo*

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