Intracellular Distribution of 5-Bis(2-chloroethyl)aminouracil-2-C<sup>14</sup> in Tissues of Tumor-bearing Rats*

Paul Byvoet and Harris Busch

(Departments of Biochemistry and Pharmacology, Baylor University College of Medicine, Houston, Texas)

SUMMARY

Rats bearing the Walker 256 carcinosarcoma were given injections intraperitoneally of 5-bis(2-chloroethyl)aminouracil-2-C<sup>14</sup>. At various time intervals following the administration of the drug, the animals were sacrificed, the tissues were excised, and the specific activities of the DNA, RNA, and the proteins of the various intracellular fractions were determined. The label of the aminouracil mustard was mainly incorporated into the RNA of the nucleus and cytoplasmic sap in the tissues studied and, to a lesser extent, into the DNA. The specific activities of the proteins were one-fourth or less than the specific activities of the RNA of the same tissues. The nuclear RNA had a higher specific activity than RNA of other cellular fractions at early time points. These data suggest that 5-bis(2-chloroethyl)aminouracil suppresses amino acid incorporation into nuclear proteins by binding to templates of protein synthesis—primarily nuclear RNA.

MATERIALS AND METHODS

The animals used in these experiments were male rats, obtained from the Holtzman Rat Company (Houston, Texas) weighing 200–250 gm. and fed Purina Laboratory Chow ad libitum. The Walker 256 carcinosarcoma was transplanted from 7 to 10 days prior to the experiment. The 5-bis(2-chloroethyl)aminouracil-2-C<sup>14</sup> was generously supplied by the Upjohn Company through the courtesy of Dr. H. G. Petering. The drug had a specific activity of 0.5 mc/m mole and was administered in solution in dimethylformamide in doses of 8 μc.—i.e., 4 mg/rat. At various time intervals following the administration of the labeled drug, the rats were anesthetized by intraperitoneal in-
Injection of 50 mg sodium pentobarbital/kg and exsanguinated by heart puncture. The tissues were rapidly excised and placed in beakers containing ice-cold saline and then immediately transferred to the cold room (4° C.), where they were homogenized in isotonic sucrose. The homogenates were subjected to differential centrifugation for the separation of the various intracellular fractions (9). The nuclei of the tumor and the liver were isolated as described recently (4). The fractions were treated consecutively with ice-cold 0.6 N PCA, ice-cold 5 per cent TCA, cold 95 per cent ethanol, followed by treatment at room temperature with absolute ethanol, chloroform-methanol (2:1), benzene, and ether (3). The dried powders, except those of the nuclei, were then extracted with 5 per cent TCA at 90° C. for 20 minutes, centrifuged, and washed with 5 per cent TCA, 95 per cent ethanol, absolute ethanol, and finally ether. The

![Chart 1](chart1.png)

**Chart 1.** Specific activity of RNA of cellular fractions of the Walker tumor at time intervals up to 6 hours following administration of aminouracil mustard-2-C\(^14\). Four mg. (8 \(\mu\)c.) of uracil mustard-2-C\(^14\) were injected intraperitoneally. At the designated time the tissues were excised from the anesthetized, exsanguinated animal and carried through the procedures indicated in the section “Materials and Methods.” The standard errors of the results at 1, 3, and 6 hours are, respectively: nuclei, 33, 32, 9, 17; mitochondria, 21, 15, 15, 23; microsomes, 25, 18, 19, 5; cytoplasmic sap, 26, 40, 35, 15.

![Chart 2](chart2.png)

**Chart 2.** Specific activity of proteins of cellular fractions of the Walker tumor at time intervals up to 6 hours following administration of aminouracil mustard-2-C\(^14\). Four mg. (8 \(\mu\)c.) of uracil mustard-2-C\(^14\) were injected intraperitoneally. At the designated time the tissues were excised from the anesthetized, exsanguinated animal and carried through the procedures indicated in the section “Materials and Methods.” The standard errors of the results at 1, 3, and 6 hours are, respectively: nuclei, 3, 2, 1, 1; mitochondria, 2, 2, 4, 5; microsomes, 2, 2, 4, 1; cytoplasmic sap, 4, 4, 8, 6.

![Chart 3](chart3.png)

**Chart 3.** Specific activity of DNA of the Walker tumor, liver, and spleen at time intervals up to 6 hours following administration of aminouracil mustard-2-C\(^14\). Four mg. (8 \(\mu\)c.) of uracil mustard-2-C\(^14\) were injected intraperitoneally. At the designated time the tissues were excised from the anesthetized, exsanguinated animal and carried through the procedures indicated in the section “Materials and Methods.” The standard errors of the results at 1, 3, and 6 hours are, respectively: tumor, 18, 15, 22, 29; liver, 37, 30, 48, 75; spleen, 14, 17, 16, 16.

**HOURS AFTER ADMINISTRATION OF AMINOURACIL MUSTARD-2-C\(^14\)**

The hot TCA extracts were plated at infinite thinness and assayed for radioactivity. The RNA content was determined by the orcinol method (9). The nuclear fractions were treated according to a slightly modified procedure of Ogur and Rosen (10): the nuclear fractions were shaken gently with 1 N PCA at 4° C. for 40 hours to extract the RNA.

1 The following abbreviations are used: TCA (trichloroacetic acid), PCA (perchloric acid), RNA (ribonucleic acid), DNA (deoxyribonucleic acid).
The extracts were neutralized with 4 N KOH, chilled to 4°C, and centrifuged at this temperature. The supernatant solutions were then plated for determination of radioactivity. The results were corrected for self-absorption by the KCIO₄ in the extracts. The precipitate left after extraction with cold 1 N PCA was treated with 5 per cent TCA at 90°C for 20 minutes to extract the DNA, centrifuged, and washed with cold 5 per cent TCA, 95 per cent ethanol, absolute ethanol, and ether. The hot TCA extracts were plated at infinite thinness and assayed for radioactivity. The DNA content was determined by means of the diphenylamine reaction (7). The RNA content was determined by the orcinol reaction; the extinction at 660 mλ due to DNA was calculated on the basis of the DNA content and was subtracted from the observed extinction. The radioactivity of the DNA was then corrected for the residual amounts of RNA which were present in small amounts in some of the extracts. The powders which were left after extraction of the various intracellular fractions with 5 per cent TCA are the protein fractions. They were plated and assayed for radioactivity as described previously (8). Each point on the graphs is the average of three to five experiments: in each experiment one rat was used.

RESULTS

Charts 1, 2, and 3 present the time course of labeling of nucleic acids and protein of the various intracellular fractions of the Walker 256 carcinosarcoma following the administration of the labeled aminouracil mustard. The specific activities of the nuclear RNA and the RNA of the cytoplasmic sap reached values up to 10 times the specific activities of the proteins at 1 hour and then declined. The specific activity of the mitochondrial RNA increased up to 3 hours after administration of the drug. The specific activity of the DNA was almost constant over the experimental period and was 4 times that of the proteins at the early time points. The specific activities of the proteins continued to increase over the period of 1–6 hours.

Charts 4, 5, and 6 present the specific activities of the nucleic acids and the proteins of the intracellular fractions of the Walker 256 carcinosarcoma.
cellular fractions of the spleen at different time intervals after the injection of the drug. In the spleen, the specific activity of the RNA of the nucleus reached values up to 20 times greater than the specific activities of the proteins at early time points. In both the spleen and the tumor the specific activities of the DNA approximated that of microsomal RNA—i.e., about 10 times the specific activity of the protein. The specific activities of the ribonucleic acids of the intracellular fractions of the spleen were the highest of the tissues studied. As in the tumor, the specific activities of the proteins continued to increase over the period of the experiment. The specific activities of the proteins of the tissues studied were more nearly equal than the specific activities of the nucleic acids.

Charts 3, 6, and 7 present the time course of labeling of the nucleic acids and proteins of the liver following the injection of the labeled aminouracil mustard. As in the other tissues studied, the specific activity of the RNA was high in the nucleus at early time points, and the maximal values were 10 times the specific activity of the proteins. In liver, the specific activity of the DNA increased up to 3 hours after administration of the aminouracil mustard-2-C14; this result was different from the result found for the spleen and the tumor.

DISCUSSION

In both tumor and spleen, the incorporation of arginine-U-C14 into nuclear proteins was markedly suppressed, whereas the uptake of uracil-2-C14 into nuclear RNA was only moderately inhibited. The data presented indicate that the drug is primarily bound to the RNA and suggest that the templates for nuclear protein synthesis are blocked by direct binding of aminouracil mustard. These data, which show that the label of the aminouracil mustard was mainly bound to the nucleic acids of the tissues studied, provide support for the concept that the "carrier" to which a nitrogen mustard is attached in part determines the intracellular distribution and binding of the drug. This view is also supported by studies carried out with amino-phenylalanine mustard-β-C14 by Cohn (6), who treated the cellular fractions with essentially the same procedures used in this study, including the extraction with hot TCA. The label of the phenylalanine mustard was mainly found in the proteins of the various intracellular fractions.

The comparatively high specific activities of the nucleic acids in the spleen may be related to the
remarkable selective action of aminouracil mustard on the spleen of tumor-bearing animals (5, 8). In a number of other studies, uptake of labeled mustards into DNA and RNA has been reported (12). The lack of correlation of uptake to biological results has been a disconcerting feature of such data. If the degree of binding of aminouracil mustard to the nucleic acids in the tissues is related to the biological effect, the question arises why protein metabolism is markedly inhibited in the Walker tumor in contrast to the liver, since the nucleic acids in both tissues take up almost equal amounts of the labeled aminouracil mustard. It is possible that the impact of the binding of nucleic acids, especially the RNA, to the aminouracil mustard differs in these tissues because of differences in mitotic rate. This would mean that cells in mitosis are more susceptible to the action of the mustard than resting cells, conceivably because nuclear and nucleolar RNA is serving different functions in dividing cells than in resting cells (11). It is also possible that the enormous regenerative capacity of the liver enables it to synthesize sufficient amounts of nucleic acids to overcome the destructive effects of the aminouracil mustard. The regenerative activity of the liver is reflected in the two- to threefold increase of the incorporation of labeled uracil into the nuclear RNA and of lysine-U-C14 into the nuclear proteins of the liver of tumor-bearing rats at 12 hours after the administration of aminouracil mustard (5, 8).

ACKNOWLEDGMENTS

The authors wish to acknowledge the very helpful assistance of Mrs. Helen R. Adams and Mr. Don Gard in certain phases of these studies.

REFERENCES

Intracellular Distribution of 5-Bis(2-chloroethyl)aminouracil-2-C14 in Tissues of Tumor-bearing Rats

Paul Byvoet and Harris Busch


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/22/2/249