Recent studies in this laboratory have demonstrated that 5-bis(2-chloroethyl)aminouracil inhibited the incorporation of frarginine-U-C\textsuperscript{14} into nuclear proteins (1, 8). In the time period studied, the incorporation of frarginine-U-C\textsuperscript{14} into the nuclear proteins of tumor and spleen was markedly inhibited, but there was little or no effect on incorporation of the label into proteins of the cytoplasmic fractions. To test the possibility that this inhibition resulted from suppression of biosynthesis of RNA, the incorporation of uracil-92-C\textsuperscript{14} into the RNA of the various intracellular fractions was determined for the Walker 9256 carcinosarcoma and spleen of tumor-bearing rats treated with the aminouracil mustard (5). The incorporation of uracil-2-C\textsuperscript{14} into the RNA was inhibited in all intracellular fractions of tumor and spleen. Since the rate of suppression of labeling of RNA was much lower than the rate of suppression of labeling of the nuclear proteins, it seemed desirable to determine whether the drug acted primarily by binding to the existing nucleic acids. With the aid of the labeled aminouracil mustard, it became possible to carry out such a study.

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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 (2). 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 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.

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 KClO₄ in the extracts. The precipitate left after extraction with cold 1 N PCA was treated with 5 per cent TCA at 90° C. for 30 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 mμ 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.
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 intratissue distribution and binding of the drug. This view is also supported by studies carried out with aminophenylalanine 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-C\(^{14}\) into the nuclear proteins of the liver of tumor-bearing rats at 12 hours after the administration of aminouracil mustard (5, 8).

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

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