The Effects of Tumor Growth, X-Radiation, and 8-Azaguanine on the Incorporation of Adenine into Nucleic Acids of Mice*

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Adenine has been shown to serve as a precursor of purine polynucleotides in the mouse (5). The ready incorporation of this compound into the nucleic acids has made labeled adenine a useful tool for the study of nucleic acid metabolism under various experimental conditions.

Tumor growth, as exemplified by the rapid proliferation of the highly cellular Sarcoma 37, is accompanied by accelerated cellular multiplication and increased DNA (desoxyribonucleic acid) synthesis. The behavior of nucleic acids during neoplasia was therefore considered worthy of observation. Because of the association of nucleic acids with chromosomes, mitosis, and tissue growth, one of the promising approaches for the control of neoplasia has been thought to be the disturbance of nucleic acid metabolism. Ionizing radiations have been shown to cause nuclear damage, chromosomal fragmentation, mutation, and inhibition of mitosis. X-radiation has been demonstrated to produce alteration of the physical-chemical properties of DNA molecules (6) and to interfere with the pattern of incorporation of nucleic acid precursors (1, 14). The carcinostatic purine analog, 8-azaguanine, has been shown to inhibit Sarcoma 37 in CAF1 mice when given in high doses (15). The close relationship in chemical structure between this analog and guanine suggested that the carcinostatic action was probably due to an interference with the metabolism of guanine. 8-Azaguanine, however, produced no effect on the direct incorporation of guanine-C14 into the nucleic acids of tumor-bearing mice (7). A competitive behavior might exist at the level of incorporation of adenine, or the analog might interfere in the interconversion of adenine and guanine.

Adenine-8-C14 was, therefore, synthesized and injected into groups of mice that (a) had been transplanted with Sarcoma 37, (b) had received x-radiation alone, (c) had been transplanted with Sarcoma 37 and had subsequently been subjected to x-radiation, (d) were receiving 8-azaguanine simultaneously, or (e) had received no other treatment, to serve as controls. Selected tissues from these animals were fractionated into the nucleic acids, and the relative specific activities of the isolated purine fractions were compared.

Preliminary results of these investigations have been presented (80).

MATERIALS AND METHODS

Synthesis.—Adenine-8-C14 was synthesized from 4,5,6-triaminopyrimidine formed by the condensation of malononitrile and thiourea, as described by Traube (29) and Hoffer (16). The triaminopyrimidine was condensed with an equimolar quantity of sodium-C14-formate in the presence of HCl. The addition of 1.3 milliequivalents of HCl, instead of 1.0 milliequivalent, as reported (10) per mole of formate, produced 4,6-diamino-5-C14-formamidopyrimidine in a yield of 95 per cent. This compound was converted to adenine-C14 by condensation with diethanolamine in an oilbath under a nitrogen atmosphere (8). Adenine was precipitated from the reaction mixture by the addition of silver nitrate to form the silver salt of the purine. The recovered adenine-8-C14 was then purified by chromatography on Dowex-50 resin. The product was found to be pure when subjected to paper chromatography in systems of n-butanol saturated

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with 1 per cent ammonium hydroxide, or tert. butanol and 8 N hydrochloric acid (7:3 v/v). Ultraviolet spectral properties of the adenine-8-C\textsuperscript{14} were identical with those reported (9). The final product had a specific activity of 0.67 μc/mg and was formed in a yield of 62 per cent based on formate-C\textsuperscript{14}.

Procedure.—Adult CAF\textsubscript{1} mice were used throughout this investigation. They were maintained on a diet of Purina Laboratory Chow and water ad libitum throughout the experiment. In each experiment the animals were divided into five groups each of 21 mice, and similar weight and sex distributions were obtained in each case. Groups A, B, and C were transplanted with Sarcoma 37. Two days after transplantation, groups A and D were exposed to 400 r of total-body x-radiation at a rate of 79 r.p.m. The animals were x-radiated with a standard "deep therapy" Picker x-ray machine. Physical factors were 200 kv., 19 ma.; 0.5 mm. copper filter; 3.0 mm. of aluminum filter; and mean animal to target distance, 28 cm. Twenty-four hours after x-radiation all mice were administered doses of 25 mg/kg of adenine hydrochloride intraperitoneally as an aqueous solution. Group B was given simultaneously intraperitoneal injections of 25 (Exp. I) or 50 (Exp. II) mg/kg of 8-azaguanine synthesized in this laboratory (21) and dissolved in 0.5 per cent sodium carbonate solution. The injection scheme was repeated at 12-hour intervals for a total of five injections each. Twenty-four hours after the last injection, the mice were killed by cervical dislocation, and the liver and tumor tissues were excised. The nucleic acids from the pooled tissues were isolated and fractionated into PNA (pentosenucleic acid) and DNA by a previously described method as described previously (11) of the procedure of Bendich et al. (4). The nucleic acid fractions BaPNA and DNA were hydrolyzed with 1 N HCl, and the purines were isolated and separated by the ion exchange chromatographic method as described previously (11). They were identified by the position of the fractions collected and by the ratio of optical densities at various wavelengths as determined in a model DU Beckman ultraviolet spectrophotometer. The purines were evaporated to dryness under reduced pressure and were washed with water. The solid was taken up in a small amount of 0.1 N HCl, and an aliquot was plated directly on a glass plate. Counting was carried out at the infinitesimal thinness level in a gas-flow proportional counter. Another aliquot was diluted with the same acid, and the concentration of the purine determined by ultraviolet spectroscopy. From these data the specific activity was calculated.

Actual incorporation into nucleic acids.—Because of the high specific activity of the injected material, it was possible that a minute amount of the injected adenine-C\textsuperscript{14} had become admixed with the excised tissues to contaminate the final adenine fraction isolated from nucleic acids. To exclude this possibility, the procedures already described (22) were followed. BaPNA was hydrolyzed to the nucleotide stage, and adenylc acid was isolated by ion exchange chromatography. This fraction was then further hydrolyzed to adenine and was radioassayed by the usual procedure. The close agreement between the specific activities of this sample and adenine obtained directly by the complete hydrolysis of BaPNA indicated that all the adenine isolated by direct hydrolysis had been in the form of a nucleotide, and none of the radioactivity of the PNA fraction was due to contaminating adenine-C\textsuperscript{14}. To establish lack of contamination of DNA, a known quantity of adenine-C\textsuperscript{14} was added to a mixture of nonactive sodium nucleates, and the fractionation into BaPNA and DNA was carried out by the usual procedure. No radioactivity was found contaminating the DNA fraction, demonstrating that all the radioactivity of the isolated DNA fractions was due to conjugated adenine, rather than contamination with the administered adenine-C\textsuperscript{14}.

RESULTS

Results are expressed in terms of relative specific activities, as per cent, which were calculated from the specific activity of the isolated purine by use of the following formula:

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\text{Relative specific activity (as per cent)} = \frac{100 \times \text{molar activity of purine isolated}}{\text{molar activity of adenine-8-C}^{14} \text{ administered}}
\]

The figures represent an estimate of the biological dilution of radiocarbon from administered adenine-8-C\textsuperscript{14} during its incorporation into nucleic acid purines and are an indication of the percentages of the isolated nucleic acid purines which were derived from the adenine-8-C\textsuperscript{14} under the experimental conditions as described. The data do not give information as to the total amount of adenine-8-C\textsuperscript{14} which was utilized for the synthesis of nucleic acid purines. The results obtained in each group of the two experiments are tabulated in Table 1.

DISCUSSION

Effect of tumor growth.—A comparison of the incorporation pattern of adenine-8-C\textsuperscript{14} into nucleic acid purines showed a higher incorporation into the livers of tumor-bearing mice than into those of...
the control group. This increase was most marked in the DNA purines, but a slight increase was also observed in the corresponding PNA fraction.

These results are in essential agreement with the findings of other investigators using different nucleic acid precursors. Kelly and Jones (17) observed that after the administration of P\textsuperscript{32} the specific activities of DNA from livers, spleens, and kidneys of mice bearing mammary carcinoma were higher than those of control mice. With C\textsuperscript{14} formate or glycine, Payne et al. (25) observed a similar result. The work of Conzelman et al. (12) employing 4-amino-5-imidazolecarboxamide is also in agreement. Kelly et al. (18) reported that, after the tumor DNA to the liver DNA fractions might account for this increase in relative specific activities. In the case of the PNA, however, the lower specific activities of the tumor purines compared with those of the liver purines would preclude the possibility of such direct transfers. Enhanced synthesis of liver nucleic acids due to an increase in the total nucleic acid content of the liver in the presence of a rapidly growing tumor might offer another explanation for the increased incorporation of the precursor. Reddy and Cercedo (27), as well as Lombardo et al. (19), have reported that the presence of a tumor increased the DNA and PNA content of livers, kidneys, and lungs of mice, as compared to that in normal animals.

The incorporation studies with adenine-C\textsuperscript{14} on CAF\textsubscript{1} mice bearing Sarcoma 37 were carried out under experimental conditions similar to those with guanine-C\textsuperscript{14} and 4-amino-5-imidazolecarboxamide-C\textsuperscript{14} previously studied in this laboratory (7, 22). A comparison of their characteristic incorporation patterns is reproduced in Table 2. The comparatively rapid turnover in the tumor and liver PNA fractions and the increased synthesis of tumor DNA presumably required for the rapid proliferation of the highly cellular neoplastic tissue might explain the higher incorporation levels in these fractions. The difference in the incorporation pattern of guanine from that of the other two precursors might be considered as evidence that the pathway of nucleic acids in Sarcoma 37 differed sharply from that in liver cells in their ability to utilize preformed guanine (20).

A large discrepancy between the ratios of relative specific activities of adenine to guanine after the administration of either adenine-C\textsuperscript{14} or 4-
aminoo-5-imidazolescarboxamide-C\textsuperscript{14} was found consistently in the various fractions. Recently, it was observed that 4-amino-5-imidazolescarboxamide was capable of inhibiting 8-azaguanine deaminase, and the carboxamide therefore potentiated the toxicity (8) as well as the carcinostatic effect (24) of 8-azaguanine. Since 8-azaguanine has been reported to be deaminated by guanase (28), it is probable that the deaminase-inhibiting property of the carboxamide might be responsible for the divergent adenine:guanine ratios after the administration of the two labeled precursors. Preliminary experiments with the simultaneous administration of adenine-C\textsuperscript{14} and 4-amino-5-imidazolescarboxamide have indicated that the incorporation of PNA guanine was increased, and the adenine:guanine ratios became comparable to those found after the administration of 4-amino-5-imidazolescarboxamide alone.

Effect of x-radiation.—The livers of normal and tumor-bearing mice after x-radiation showed slightly increased incorporation of adenine into the liver PNA purines and a definitely decreased incorporation into the liver DNA purines. These results paralleled those of Payne et al. (26) and Conzelman et al. (12), who used P\textsuperscript{32} and 4-amino-5-imidazolescarboxamide-C\textsuperscript{14}, respectively, as precursors. Other investigators have found that x-radiation inhibits the synthesis of DNA (15).

There was a slight increase in incorporation of adenine into the tumor PNA purines of x-irradiated mice compared with the controls, while the incorporation into tumor DNA purines was essentially the same. This lack of effect of x-radiation on the relative specific activities of DNA purines in Sarcoma 37 was surprising. One might expect that such a rapidly growing cellular tumor would show more marked changes, after x-radiation, in the incorporation into DNA than would a relatively nongrowing adult tissue such as the liver. It is possible that the recovery periods of the various tissues from the effects of x-radiation show wide time variations and that the DNA metabolism of the tumor had recovered sufficiently from the ionizing radiations in the 24 hours before the adenine administration so that an effect could no longer be demonstrated. The experimental conditions of studies of this nature must be evaluated carefully before results of different investigators can be compared.

Effect of 8-azaguanine.—The simultaneous administration of 8-azaguanine and adenine-C\textsuperscript{14} produced no decisive effect on the pattern of incorporation of adenine-C\textsuperscript{14} into tumor and liver nucleic acid purines. Abrams (2) has reported that 8-azaguanine was completely without effect on the utilization of glycine and adenine for the synthesis of intestinal nucleic acid purines in the Sprague-Dawley rat. Previous studies carried out in this laboratory (7) similarly had demonstrated no effect of 8-azaguanine on the incorporation of guanine-C\textsuperscript{14}. Even though the dosage of 8-azaguanine was subinhibitory, a definite effect on the incorporation pattern of the various purine precursors was expected if the analog antagonized the particular biochemical pathway under investigation. Due to the potentiating effect of 4-amino-5-imidazolescarboxamide-C\textsuperscript{14} (8), slight tumor inhibition was produced when this substance was administered with 8-azaguanine; nevertheless, the incorporation pattern of the precursor remained unchanged in the presence of the analog (7). In view of the incorporation of 8-azaguanine into the nucleic acid fractions of the tumor and liver cells (23), it is more likely that the analog owes its carcinostatic action to the formation of an 8-azaguanine-containing polynucleotide unable to carry out its normal functions than to an interference in the direct incorporation of purines (20).

SUMMARY

1. Adenine-8-C\textsuperscript{14} was synthesized from 4,5,6-triaminopyrimidine and sodium C\textsuperscript{14}-formate and was injected intraperitoneally into CAF\textsubscript{1} mice.
2. The effects of Sarcoma 37, x-radiation, and the simultaneous administration of 8-azaguanine on the incorporation of adenine-C\textsuperscript{14} into the nucleic acids of tissues from the mice were evaluated.
3. In tumor-bearing mice a high preferential incorporation into the liver PNA purines and a lesser but similar incorporation into the tumor PNA and DNA fractions were observed.
4. The relative specific activities of liver DNA purines were higher, and the PNA purines were slightly higher in tumor-bearing than in control mice.
5. X-radiation produced a decreased incorporation into liver DNA purines and a slightly increased incorporation into the PNA purines of the livers of normal as well as tumor-bearing mice.
6. X-radiation produced a slightly increased incorporation into the tumor PNA but no appreciable change in the tumor DNA purines of tumor-bearing mice.
7. The simultaneous administration of 8-azaguanine and adenine-C\textsuperscript{14} produced no effect on the pattern of incorporation of adenine into the PNA or DNA.

REFERENCES

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