Studies on the Distribution of Radioactive 8-Azaguanine (Guanazolo) in Mice with Eo771 Tumors*†

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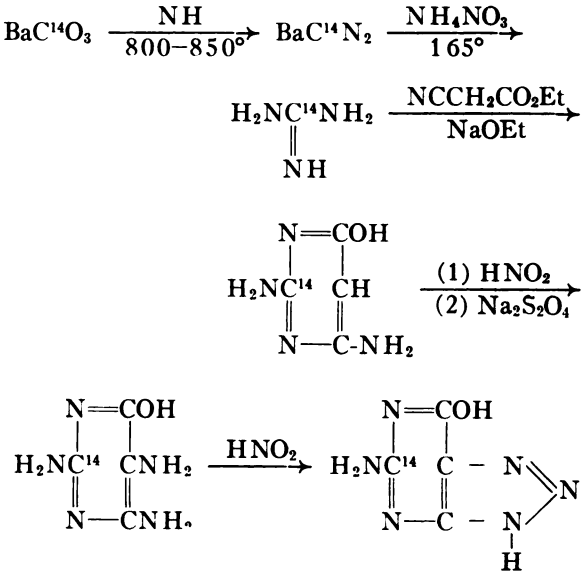
Kidder's observation (7) that 8-azaguanine (guanazolo) has a definite inhibitory effect on the growth of mammary adenocarcinoma Eo771 has now been extended and confirmed (5, 12). However, it has been found that Sarcoma 180, lymphosarcoma 6C3HED, sarcoma T241, Harding-Passey melanoma, Wagner osteogenic sarcoma or Patterson lymphosarcoma in the mouse, and certain carcinomas and sarcomas in the rat fail to respond to this compound (5, 12). 8-Azaguanine has also been shown to effect an inhibition of two transplantable leukemias in mice (8), while other strains failed to respond (1, 8). Kidder *et al.* (7) suggested that the tumor-inhibiting activity of 8-azaguanine might be explained on a basis of preferential incorporation of this compound into cancer cells because of differences in guanine metabolism. Roblin *et al.* (10) found 8-azaguanine to be a potent purine inhibitor in *Escherichia coli* and *Staphylococcus aureus*. Kidder (6) has reported a similar inhibition of growth in *Tetrahymena geleii*.

In view of the importance of such observations as have been mentioned above, it was considered worth-while to investigate the tumor-inhibiting mechanism of the action of 8-azaguanine, by use of the carbon 14-labeled compound. The synthesis of 8-azaguanine-2-C^{14} was accomplished by minor modifications of established synthetic methods, as follows. The final product was characterized by ultraviolet absorption spectrum, elemental analysis, and filter paper chromatography combined with autoradiography.



The present study concerns the over-all distribution of the 2-carbon atom of 8-azaguanine at various periods after injection into both normal mice and mice bearing a tumor known to respond —Eo771 adenocarcinoma. Further investigation of Kidder's hypothesis regarding preferential entrance of this purine antagonist into nucleic acids of tumor cells is being reported in the next article of this issue (9).

EXPERIMENTAL

SYNTHESIS OF RADIOACTIVE 8-AZAGUANINE

Guanidine hydrochloride.—Barium carbonate (3 mc., 168 mg.) was converted to guanidine hydrochloride essentially as described by Marsh, Lane, and Salley (3). The crude product (47 mg.) was diluted to 1 gm. with inactive guanidine hydrochloride and was used directly in the next step.

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2,4,5-Triamino-6-hydroxypyrimidine.—Guanidine hydrochloride (1 gm.) was converted to 2,4,5-triamino-6-hydroxypyrimidine by the procedure of Cain, Mallette, and Taylor (2), with slight modifications. The pyrimidine was isolated as the sulfate; the yield was 0.65 gm., 24 per cent from guanidine. In subsequent syntheses, this pyrimidine has been obtained in a 70–75 per cent yield from guanidine and in a 40–59 per cent over-all yield from barium carbonate.

8-Azaguanine.—The procedure of Roblin *et al.* (10) was used for conversion of 2,4,5-triamino-6-hydroxypyrimidine to 8-azaguanine. The crude product, after being twice dissolved in sodium hydroxide, treated with charcoal, and reprecipitated with acetic acid, weighed 257 mg. and had an activity of 0.97 μc/mg (67 per cent yield from 2,4,-5-triamino-6-hydroxypyrimidine; over-all yield from barium carbonate, 8.3 per cent).

Analysis: Calculated for $C_4H_4ON_6$: C, 31.58; H, 2.65
Found: C, 31.47; H, 2.82

The ultraviolet absorption spectrum, determined on a solution of 3.7 mg/l in phosphate buffer of pH 6.30, was identical with that reported by Cavalieri *et al.* (4).

As a further check on purity, the sodium salt was chromatographed (ascending) on filter paper with the following results:

Solvent System	R_F values Found	Reported*
n-C_4H_9OH (4 parts), diethylene glycol (1 part), H_2O (1 part) in an NH_3 atmosphere	0.11	0.13
n-C_4H_9OH (4 parts), diethylene glycol (1 part), 2 N HCl (1 part)	0.42	0.42–0.46

* A. Bendich, Sloan-Kettering Institute for Cancer Research. Private communication.

In an independent experiment the sodium salt was chromatographed, and an autoradiograph was made of the filter paper strip. The position of the radioactive spot coincided exactly with the position of the 8-azaguanine as determined in ultraviolet light; there was no detectable radioactivity elsewhere on the filter paper.

INJECTION OF RADIOACTIVE 8-AZAGUANINE

In a preliminary experiment, two normal CFW strain mice of about 6 weeks of age were each injected with 1.93 μc. of labeled 8-azaguanine (80 mg/kg), made up in 1 per cent sodium carbonate. These animals were placed immediately into a metabolism chamber (11) for collection of expired carbon dioxide and excreta. After 24 hours, the mice were killed, and selected pooled tissues and organs were assayed for radioactivity. The data obtained in this experiment with regard to excretion and distribution of radioactive carbon are summarized in Tables 1 and 2.

TABLE 1

RATE OF EXPIRATION AND URINARY EXCRETION OF C^{14} FOLLOWING INJECTION OF LABELED 8-AZAGUANINE (1.93 μc.)

Period	Specific activity of samples (μc/mole carbon)*	Per cent of total injected radioactivity	Cumulative per cent of total injected radioactivity
0–30 minutes	0.644	0.06	0.06
30–60 minutes	0.663	0.08	0.14
1–2 hours	0.096	0.02	0.16
2–6 hours	0.015	0.01	0.17
6–12 hours	0.142	0.03	0.20
12–24 hours	0.107	0.15	0.35
0–24-hour urine sample	321.0	91.4	

* Microcuries per mole of carbon.

NOTE: These are the average data obtained on pooled respiratory samples from two normal CFW strain mice.

TABLE 2

GENERAL DISTRIBUTION OF RADIOACTIVE CARBON AT VARIOUS PERIODS AFTER INJECTION OF 2-LABELED 8-AZAGUANINE

SPECIFIC ACTIVITY (μc/MOLE OF CARBON)*

Tissue	Normal CFW strain mice 24 hrs. after injection	C57 black mice with Eo771 Tumors Period after injection 1 hr.	6 hrs.	24 hrs
Whole blood	0.012	0.392	0.183	0.016
Blood serum	0.099			
Blood cells	0.016			
Spleen	0.079	0.776	0.467	0.131
Adrenals		0.389	0.125	0.012
Kidneys	0.086			
Liver	0.041	0.503	0.350	0.078
Testes	0.036			
Thymus	0.054			
Lungs	0.025			
Heart	0.056			
Lymph nodes	0.037			
Muscle	0.008	0.828	0.030	0.019
Jejunum	0.157	1.170	0.344	0.306
Skin and hair	0.018			
Whole bone	0.036			
Bone marrow	0.089	0.594	0.129	0.028
Eo771 tumor (animal 1)		0.861	0.130	0.078
Eo771 tumor (animal 2)		0.432	0.167	0.074
Urine	321.0			
Feces	8.15			

* Microcuries per mole of carbon.

A second series of investigations was then undertaken to determine the distribution of carbon 14 from labeled 8-azaguanine in C57 black mice with the Eo771 adenocarcinoma. Subcutaneous implantations of small pieces of tumor (measuring about 1.5 mm. cubed, and weighing approximately 6 mg.) were made in the axillary region by the usual trocar method. After 8 days,

two of these tumor-bearing mice were each injected with 1.93 μc. of radioactive 8-azaguanine. One hour after the injection of the radioactive compound, these mice were sacrificed for tissue and organ activity assays. The tissues of interest from the two mice used in each of these experiments were pooled, except in the case of the tumors which were run separately. Two additional mice (9 days after tumor implantation) were injected at the same level of labeled 8-azaguanine and were sacrificed for distribution studies at 6 hours. A similar study was carried out on two Eo771 tumor-bearing mice (9 days after implantation), in which mice were sacrificed for activity studies 24 hours after injection of the labeled compound. The results obtained in these experiments are presented in Table 2.

DISCUSSION

The results presented in Table 1 indicate the route of excretion of the 2-carbon atom of 8-azaguanine. Since only about 0.35 per cent of the total injected radioactive carbon was found in the exhaled carbon dioxide, it appears that there was no extensive oxidation of the molecule. Most of the activity injected (91.4 per cent) can be accounted for in the urine within 24 hours.

The distribution of the active carbon atom in CFW mice at 24 hours shows more activity in the blood serum than in packed cells and the highest specific activity in the jejunum. Otherwise, the active carbon is generally found in all tissue fractions.

In the Eo771 tumor-bearing C57 black mice the specific activities of the tumors fall within the range covered by the other tissues. The jejunum is again the highest in radioactivity, in all cases being higher than the tumor tissue from the same animals. Although the present data do not indicate preferential accumulation of 8-azaguanine in neoplastic tissue, the possibility obviously exists that 8-azaguanine might be preferentially fixed to or incorporated into important fractions of cancer cells, i.e., nucleic acids, which might be masked in these gross observations. This point has received special attention in experiments reported in the following article (9).

SUMMARY

Studies with 2-labeled 8-azaguanine (guanazolo) in normal and mammary adenocarcinoma-bearing mice have shown that very little of the 2-carbon atom is oxidized to carbon dioxide and exhaled, but that most of this atom is excreted in the urine. No evidence was obtained that the compound in question (or the 2-carbon therefrom) was preferentially accumulated, fixed, or incorporated in Eo771 tumors, which are known to be inhibited by 8-azaguanine.

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