THE EFFECT OF 4-AMINO-5-IMIDAZOLECARBOXAMIDE ON THE INCORPORATION OF PURINES INTO LIVER NUCLEIC ACIDS OF THE MOUSE*

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In recent experiments in which 8-azaguanine was administered simultaneously with 4-amino-5-imidazolecarboxamide (AIC) to mice, the toxicity of the purine analog was greatly potentiated[1], and the carcinostatic potency of 8-azaguanine was similarly enhanced[2]. Further experiments on deamination with liver homogenates demonstrated an inverse relationship between the concentration of AIC and the extent of deamination of 8-azaguanine. The conclusion was drawn that AIC, like various pteridine compounds[3], inhibited the enzyme which normally deaminated 8-azaguanine to the urinary metabolite, 8-azaxanthine[4], a relatively non-toxic and non-carcinostatic catabolic product[5].

The *in vitro* ability of guanase to deaminate guanine and azaguanine[6] has suggested that the same enzyme is involved in both reactions *in vivo*. If AIC inhibits this enzyme, one would expect changes in the metabolic rate of administered guanine, particularly a decrease in the catabolism of this purine. As a result, more of this purine should be utilized for nucleic acid synthesis, since normally guanine is incorporated into mouse polynucleotides to a small though definite extent[7].

For the present investigation, mice were injected with guanine-^{14}C plus AIC, or with guanine-^{14}C only, and specific activities of liver nucleic acid purine fractions were compared. Similar experiments on the coadministration of adenine-^{14}C and AIC also were carried out. A preliminary report of these investigations has been presented[8].

METHODS

CAF_1 mice*** of both sexes were used in equal numbers. Guanine-^{14}C, synthesized in this laboratory[7], was dissolved in 0.5 *N* NaOH solution and was neutralized with HCl to form a fine suspension prior to injection. AIC hydrochloride[9] was neutralized with sodium carbonate prior to administration. Adenine-8-^{14}C, prepared as previously reported[10], was used as the hydrochloride. Compounds were administered intraperitoneally every twelve hours until five doses of the various drugs or combinations had been injected. The animals were killed by cervical dislocation 24 hours after the last injection. Doses of compounds per injection were: guanine, 25 mg per kg; adenine, 25 mg per kg, and AIC, 25 or 50 mg per kg, as indicated in Table I.

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The method of CONZELMAN *et al.*[9] was used for the isolation and degradation of nucleic acids from mouse livers. The homogenized tissue was dried, extracted with 10% NaCl solution, and the combined nucleic acids were precipitated with alcohol. After the separation[11] into pentose-nucleic acid (PNA) and deoxypentosenucleic acid (DNA) they were hydrolyzed to the purine bases. The PNA and the DNA purine bases were separated by ion exchange chromatography[12] on Dowex-50. Specific activities were determined from radioactivity counts of aliquots in a proportional gas-flow counter and from ultraviolet absorption measurements in a Beckman DU spectrophotometer. Percent of relative specific activities (RSA) were calculated according to the formula,

$$\text{RSA} = 100 \times \frac{\text{molar activity of isolated purine}}{\text{molar activity of administered purine}}$$

RESULTS AND DISCUSSION

The results are presented in Table I. It is apparent that the presence of AIC enhanced the RSA of the guanine fractions of both DNA and PNA after the administration of guanine-^{14}C. In the case of PNA a five-fold increase was observed. No increase was noted in the corresponding adenine fractions, although the low extent of conversion to this purine makes these values subject to considerable error. As indicated, AIC did not influence significantly the distribution of radioactivity of the nucleic acid purines derived from adenine-^{14}C.

TABLE I

RELATIVE SPECIFIC ACTIVITIES, IN %, OF LIVER NUCLEIC ACID PURINES AFTER THE ADMINISTRATION OF ISOTOPIC PURINES IN THE PRESENCE AND ABSENCE OF 4-AMINO-5-IMIDAZOLECARBOXAMIDE

		Relative specific activities			
		PNA		*DNA*	
^{14}C Purine	*AIC*				
25 mg per kg	*mg per kg*	*adenine*	*guanine*	*adenine*	*guanine*
Guanine	0	0.02	0.26	$<$0.01	0.07
Guanine	0	0.02	0.25		
Guanine	0	0.02	0.28		
Guanine	50	0.01	1.10	$<$0.01	0.13
Guanine	50	0.03	1.31		
Guanine	50	0.04	1.21		
Adenine	0	6.50	0.90	0.13	0.02
Adenine	0	6.50	0.94		
Adenine	0	6.45	0.85		
Adenine	25	6.50	0.95	0.17	0.04
Adenine	25	6.55	0.98		
Adenine	50	6.25	1.01		
Adenine	50	6.25	1.01		
Adenine	50	6.07	0.95		

It would seem, therefore, that guanase is inhibited by AIC, thus decreasing the catabolic breakdown of exogenously supplied guanine-^{14}C, and allowing more of the purine to be incorporated into the nucleic acids. Since the purine interconversion apparently does not occur at the level of the bases but at a later stage in biosynthesis[13], no appreciable increase in the RSA of the adenine fractions should be observed. Sim-

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ilarly, after the administration of adenine-^{14}C the conversion to guanine-containing moieties of nucleic acids does not involve predominantly guanine *per se*, and therefore the coadministration of AIC did not produce a significant effect on the incorporation pattern of adenine into nucleic acids.

SUMMARY

In investigations in which guanine-^{14}C was injected into mice, the relative specific activities of the guanine fractions isolated from liver nucleic acids were increased approximately five-fold when 4-amino-5-imidazolecarboxamide (AIC) was co-administered. It was concluded that AIC inhibited guanase, thus blocking the catabolism of guanine. The potentiation of 8-azaguanine actions by AIC previously reported is apparently caused by inhibition of the same enzyme. No significant effect of AIC on the metabolism of adenine-^{14}C was observed.

REFERENCES

1 P. E. CARLO AND H. G. MANDEL, *Cancer Research*, 14 (1954) 459.
2 H. G. MANDEL AND L. W. LAW, *Cancer Research*, 14 (1954) 808.
3 L. S. DIETRICH AND D. M. SHAPIRO, *J. Biol. Chem.*, 203 (1953) 89.
4 H. G. MANDEL, E. L. ALPEN, W. D. WINTERS AND P. K. SMITH, *J. Biol. Chem.*, 193 (1951) 63.
5 E. HIRSCHBERG, J. KREAM AND A. GELLHORN, *Cancer Research*, 12 (1952) 524.
6 A. ROUSH AND E. R. NORRIS, *Arch. Biochem.*, 29 (1950) 124.
7 H. G. MANDEL AND P. E. CARLO, *J. Biol. Chem.*, 201 (1953) 335.
8 J. L. WAY AND H. G. MANDEL, *Federation Proc.*, 14 (1955) 300.
9 G. M. CONZELMAN, Jr., H. G. MANDEL AND P. K. SMITH, *J. Biol. Chem.*, 201 (1953) 329.
10 J. L. WAY, H. G. MANDEL AND P. K. SMITH, *Cancer Research*, 14 (1954) 812.
11 G. SCHMIDT AND S. J. THANNHAUSER, *J. Biol. Chem.*, 161 (1945) 83.
12 W. E. COHN, *Science*, 109 (1949) 377.
13 R. ABRAMS AND M. BENTLEY, *Arch. Biochem. Biophys.*, 56 (1955) 184.

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ATPASE ACTIVITY OF RAT LIVER MITOCHONDRIA

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The rate at which mitochondria hydrolyse adenosine triphosphate (ATP) is known to be variable. Thus mitochondrial ATPase may be inactive, *e.g.* in mitochondria prepared in sucrose (KIELLEY AND KIELLEY[1]; LARDY AND WELLMAN[2]; POTTER, SIEKEVITZ AND SIMONSON[3]). On the other hand mitochondria prepared in a saline medium can hydrolyse ATP at a rate which is approximately balanced by the rate of ATP formation by oxidative phosphorylation. Under these conditions the rate of incorporation of radioactive inorganic phosphate (^{32}P) into ATP measures both the rate of oxidative phosphorylation (KREBS, RUFFO, JOHNSON, EGGLESTON AND HEMS[4]) and the rate of ATPase activity. ATPase activity may also be increased by extraneous agents *e.g.* by DNP (HUNTER[5]). This paper is concerned with the activity of mitochondrial ATPase under various conditions and with its relation to oxidative phosphorylation.

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