Effect of Nitrogen Mustard on Nucleic Acid Metabolism.* (19670)

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Histological, biological and biochemical evidence(1) has been interpreted to implicate nucleic acid metabolism as a possible locus of action of nitrogen mustards. Studies in the rat have demonstrated a decrease in the incorporation of P^{32} into nucleic acids under the influence of the drug(2), while C^{14}-formate incorporation into nucleic acids of the mouse is also depressed(3). Since there was evidence that formate was incorporated into the 4-amino-5-imidazole-carboxamide ribotide (4,5) and that adenine was incorporated into nucleic acids without opening of the ring at the 2 position(6)‡ the hypothesis that free adenine enters the pathway of nucleic acid synthesis after the carboxamide ribotide step appeared logical. On this basis, if a drug affected any of the early steps in the synthesis of the purine ring, a decrease in the incorporation of a simple precursor such as formate would not be associated with a similar decrease in the more complex molecule adenine when both precursors were administered simultaneously. Indeed, aminopterin has been shown to produce a differential effect on formate incorporation compared to adenine incorporation and the problems involved in the experiment have been discussed(7). However, in the following experiment, nitrogen mustard produced similar decreases in the incorporation of N^{15}-adenine and of C^{14}-formate into the adenine of DNA (desoxypentose nucleic acid) of the small intestine of the rat.

Material and methods. Two groups of 10 Sherman strain 200 g rats were injected by intraperitoneal route simultaneously with isotopic formate solution§ (0.014 mM per kilo or 7.6×10^7 counts per minute per kilo as determined by gas flow counter), and N^{15}-adenine (4.83 atom % excess N^{15} and in the 1 and 3 positions(8) made up in solution with 2 meq. of lactic acid per meq. adenine and administered 0.2 mM per kilo). Fifteen minutes prior to the administration of the isotopic precursors, the rats of one group each received a single intravenous injection of nitrogen mustard (bis(2 chloroethyl) methylamine hydrochloride, 0.4 mg per ml in 0.9% NaCl, dose 4 mg per kilo), which is approximately 2 to 4 x the LD50 dose(1).

All animals were sacrificed 24 hours after injection and the tissues immediately frozen. Free bases of DNA of small intestine and PNA (pentose-nucleic acid) of small intestine and liver were separated by a column chromatographic procedure and their isotopic levels determined as described elsewhere(7).

Results and discussion. The results of the isotope determinations are presented in Table I. Nitrogen mustard does not appear to have a differential effect on the level of C^{14}-formate as compared to that of N^{15}-adenine. The change in the C^{14} activity in the adenine of the DNA of small intestine was 58%, while the change in N^{15} due to the drug was 75%. Thus the parallel depression of both precursors suggests that nitrogen mustard does not affect specifically any of the steps leading to the synthesis of the purine skeleton.

The second point of interest is the preferential effect of nitrogen mustard on the incorporation of the isotopes into DNA as compared to PNA. Among the purine bases isolated from PNA of liver and intestine, the liver adenine fraction was the only one which showed any decrease in isotope level following drug administration. These results corroborate other evidence which suggests a preferential effect of nitrogen mustard on

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§ Obtained from the Oak Ridge National Laboratory.

TABLE I. Effect of Nitrogen Mustard on Incorporation of C^{14}-Formate and N^{15}-Adenine into Nucleic Acids of Intestine and Liver.

		C^{14} activity, c.p.m./μM			N^{15} atom % excess		
		Control	Mustard	% change	Control	Mustard	% change
				Intestine			
DNA	Adenine	182	77	−58	.089	.022	−75
	Guanine	464	175	−62	.024	.004	−83
	Thymine	461	195	−58			
	Cytosine	10	11	—			
PNA	Adenine	256	259	0	.139	.153	+10
	Guanine	329	330	0	.035	.047	+34
	Uracil	15	14	—			
	Cytosine	9	14	—			
				Liver			
PNA	Adenine	57	47	−18	.139	.127	− 9
	Guanine	25	39	+56	.041	.044	+ 7
	Uracil	9	8	—			
	Cytosine	7	12	—			

DNA, as opposed to PNA metabolism. When P^{32}-phosphate was used as a precursor of the nucleic acid of bone marrow, spleen and thymus, the depression of incorporation into DNA was more marked than into PNA(2) following mustard treatment. The tissue content of DNA was decreased to a greater extent than that of PNA by nitrogen mustard in both the amblystoma embryo(9) and *Escherichia coli*(10). Finally, viruses with a high DNA content were more easily inactivated by nitrogen mustard than viruses with a high PNA content, while pneumococcus transforming factor, a DNA preparation, showed extreme sensitivity(11).

It should be emphasized that the biological and chemical evidence which implicates nucleic acid metabolism (particularly DNA) as the locus of action of nitrogen mustards *in vivo* is indirect. Knowledge regarding reduplication of particles or cells is as yet so meager that such *in vivo* experiments cannot distinguish between a specific effect of the drug on nucleic acid metabolism and an effect of the drug on other portions of the system involved in reduplication.

Summary. 1. The effect of nitrogen mustard on the incorporation of C^{14}-formate and N^{15}-adenine into the bases of PNA and DNA of small intestine and into the bases of PNA of liver of the rat has been investigated. 2. The drug decreased the incorporation of both precursors into adenine of DNA of intestine to approximately the same extent. This suggests that the drug does not affect specifically the synthesis of the purine skeleton. 3. The drug led to a marked decrease of isotope levels in the bases of DNA of small intestine, but little or no change in the levels in PNA bases of both small intestine and liver.

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