be determined not only by the total quantity of cholesterol, but also by the quantity of ATP and of coenzyme A. This hypothesis was confirmed by measuring ACAT activity in different parts of the human aorta in the intima, media, and adventitia, using palmityl-[$^{14}$C]-CoA as the substrate. As Table 1 shows, ACAT activity in the human aorta, when labeled cholesterol was given, did not exceed 27 pmoles/mg protein·h, or 27 conventional units (c.u.). When labeled palmityl-CoA was used, ACAT activity was sharply "increased" and ranged from 250 to 400 c.u. in different parts of the intima, from 300 to 880 c.u. in the media, and between 100 and 970 c.u. in the adventitia. In parallel experiments, in the same areas activity of the enzyme hydrolyzing cholesterol esters was determined, using cholesteryl-palmitate as the substrate. The hydrolyzing activity varied from 1 to 3 c.u. in the intima, from 1.4 to 3.6 c.u. in the media, and from 2.0 to 4.6 c.u. in the adventitia. All the data given above relate to the adult human aorta. The aortas of children had appreciably higher ACAT activity than those of adults, although activity of cholesterol esterase in this case showed little change (Table 3).

The approach to the study of the role of enzymes synthesizing and hydrolyzing cholesterol esters in the processes of atherogenesis by seeking factors activating or inhibiting these enzymes, but without allowing for the whole range or, at least, the principal components which determine enzymic activity at a given moment, in the given area of the vessel wall, is thus technically incorrect.

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# MECHANISM OF METHYLATION OF DNA BASES BY SYMMETRICAL DIMETHYLHYDRAZINE


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The effect of disulfiram on alkylation of the purine bases of DNA in the liver and intestine of rats was studied during the action of 1,2-dimethylhydrazine-$^{3}$H (DMH). Preliminary administration of disulfiram, which inhibits N-oxidation of DMH, prevents methylation of the guanine of DNA. Under these circumstances, however, radioactivity is incorporated into normal (non-methylated) purine bases in the course of their synthesis. The absence of methylation of DNA during the action of disulfiram is evidence that it is alkylated by the carbonium ion and not by the methyl radical.



KEY WORDS: metabolism of 1,2-dimethylhydrazine; disulfiram; alkylation of DNA.


1,2-Dimethylhydrazine (DMH), which selectively induces intestinal neoplasms [3], leads to methylation of DNA in various tissues [1, 8, 12]. DMH metabolism begins with its dehydrogenation to azomethane [4]. The conversion of azomethane can take place either through N-oxidation with the formation of methylazoxymethane or by α-C-hydroxylation with conversion into methylazomethanol. In the first case, as a result of the decomposition of methylazoxymethanol a carbonium ion ($CH_3^+$) is formed, whereas in the second case it would be expected that a methyl radical ($C^{\bullet}H_3$) would be formed as a result of the homolytic breakdown of methyldiimine. However, these terminal particles, which differ in their chemical nature and reactivity, can interact differently


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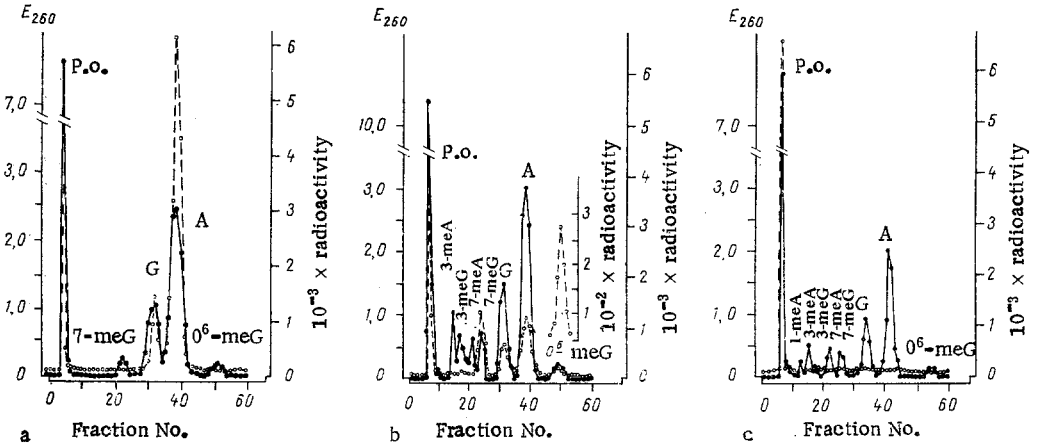


Fig. 1. Radiochromatogram on Sephadex G-10 column of hydrolysate of DNA from mucous membrane of rat large intestine: a) group 1; b) group 2; c) group 3. Broken line represents radioactivity (in cpm/fraction), continuous line optical density ($E_{260}$). P.o.) Pyrimidine oligonucleotides; 1-meA) 1-methyladenine; 3-meA) 3-methyladenine; 7-meA) 7-methyladenine; 3-meG) 3-methylguanine; 7-meG) 7-methylguanine; G) guanine; A) adenine; $O^6$-meG) $O^6$-methylguanine.

with biopolymers. It has been suggested that the carcinogenic action of DMH is due to methylation of information macromolecules with the carbonium ion only [4].

It has recently been shown that disulfiram, which completely prevents the carcinogenic effect of DMH [13], blocks the N-oxidation of azomethane [6], so that it is impossible for the carbonium ion to be formed [4, 5, 14].

The facts described above, together with theoretical assumptions, enable the mechanism of methylation of DNA by DMH metabolites to be studied by using the ability of disulfiram to block penetration of the carbonium ion.

## EXPERIMENTAL METHOD

Experiments were carried out on 15 noninbred male rats. For 8 days 5 rats (group 1) were given disulfiram (Teturam) in sunflower oil in a dose of 5 g/kg body weight daily through a tube. Four hours after the last introduction of disulfiram the rats were given a subcutaneous injection of a neutral solution of tritiated DMH·2HCl (specific activity 14.2 mCi/mmole) in a dose of 3 mCi/kg. The dose of the label was adjusted to 21 mg/kg, calculated as base, by the addition of unlabeled DMH. Four hours after injection of the DMH-$^3$H a further dose of sulfiram was given, sufficient to completely prevent N-oxidation of the carcinogen. Another 5 rats (group 2) received a subcutaneous injection of [$^3$H]-DMH, whereas the remaining 5 animals (group 3) received [$^3$H]-$H_2O$ in the same dose, without any preliminary procedures.

The rats were killed by cervical dislocation 9 h after receiving the labeled DMH or water, and the liver and mucous membrane of the large intestine were frozen in liquid nitrogen. DNA was extracted from the tissues by the phenol method. The purine bases were separated chromatographically in a Sephadex G-10 column and their radioactivity determined in a Mark II scintillation counter (Nuclear Chicago, USA) by the method of Margison et al. [10, 11].

## EXPERIMENTAL RESULTS

As Table 1 shows, the 7-methylguanine level in DNA of the mucous membrane of the large intestine of the animals receiving DMH (group 2) was only one-fifth of that in the liver DNA. Meanwhile the $O^6$-methylguanine level in the liver DNA was only twice as high as in the DNA of the large intestine. As a result the $O^6$-methylguanine: 7-methylguanine ratio in the DNA of the large intestine was twice as high. By contrast with these observations, in the rats receiving disulfiram before DMH (group 1), no alkylation of purine took place.

The chromatogram of the purine bases of DNA in the mucous membrane of the large intestine of the rats demonstrated absence of alkylated purine in animals receiving sulfiram before injection of DMH and also in animals receiving labeled water only. However, by contrast with the rats of group 3, intensive incorporation of radioactivity into adenine and guanine was observed in the rats of group 1 (Fig. 1).

TABLE 1. Levels of 7-Methylguanine and $O^6$-Methylguanine in DNA of Liver and Mucous Membrane of Large Intestine of Rats Receiving DMH

| Experimental conditions | Organ | Quantity of methylated guanine, cpm/μ mole guanine | | Ratio $O^6$-methylguanine: 7-methylguanine |
|---|---|---|---|---|
| | | 7-methylguanine | $O^6$-methylguanine | |
| Administration of disulfiram and DMH (group 1) | Liver | 0 | 0 | — |
| | Large intestine | 0 | 0 | — |
| Administration of DMH (group 2) | Liver | 3237 | 355 | 0,11 |
| | Large intestine | 664 | 141 | 0,21 |

The results indicate that the ratio $O^6$-methylguanine: 7-methylguanine in DNA of the mucous membrane of the large intestine, an organ with affinity for DMH, 9 h after injection of DMH was increased almost four-fold compared with its value 3 h after administration of this carcinogen [1]. These results thus agree with the view that the formation of $O^6$-methylguanine lies at the basis of the carcinogenic action of alkylating compounds, for persistence of this "promutagenic" base in the DNA molecule increases the likelihood of non-complementary base pairing during DNA replication [7, 9, 11].

Incorporation of radioactivity into normal (nonmethylated) purine bases of rats with blocked N-oxidation, i.e., in animals receiving disulfiram before administration of DMH, indicates that the methyl group of DMH is used in their synthesis. Radioactive label was evidently incorporated into adenine and guanine from the formate formed from formaldehyde either as a result of heterolysis of methylazomethanol into methyldiimine or by further oxidation of the latter compound. The control experiments, in which no label was incorporated either into adenine or into guanine after administration of labeled water to the rats, confirmed this conclusion.

In the rats which received disulfiram as well as DMH, incorporation of radioactivity into the purine bases in the course of their synthesis was much more intensive than in animals receiving DMH alone (Fig. 1). This could indicate slowing of the total destruction of DMH under these conditions and the greater degree of utilization of the formaldehyde formed in the course of its metabolism in purine synthesis, as is confirmed by the considerable reduction in $CO_2$ formation during such experiments [6].

As was observed previously [4], DMH metabolism, proceeding through α-C-hydroxylation, predetermines the formation of a methyl radical. In the experiments with disulfiram, ruling out the possibility of N-oxidation of DMH metabolites, a methyl radical is evidently formed, but methylation of the purine bases of DNA does not take place under these circumstances. The investigations thus demonstrate that only the carbonium ion has a methylating effect on DNA bases.

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