either the combined secretion of NaCl or secretion of Cl^- only [11]. As a result of these processes NaCl accumulates in the lumen of the intestine in cholera intoxication. Inhibition of K,Na-activated adenosine triphosphatase also evidently takes place, with the consequent loss of intracellular potassium [5].

The action of cholera toxin in the stage of the developed syndrome of rapid intestinal dehydration thus causes marked changes in electrolyte transport and also in the ultrastructure of the microvilli of the enterocytes. All these changes are focal in character, with their predominant localization on the apical part of the villi.

LITERATURE CITED

1. A. P. Avtsyn, V. A. Shakhlamov, and O. F. Sageeva, Arkh. Patol., No. 3, 41 (1973).
2. T. G. Barkhina and M. N. Lyapin, Byull. Éksp. Biol. Med., No. 4, 478 (1978).
3. H. Brockerhoff and R. G. Jensen, Lipolytic Enzymes, Academic Press, New York (1974).
4. S. V. Buravkov, V. I. Sorokovoi, and V. A. Shakhlamov, in: Proceedings of the 11th All-Union Conference on Electron Microscopy [in Russian], Tallin (1979), p. 86.
5. A. V. Gor'kova, K. M. Mokhin, L. G. Belov, et al., Problems in Especially Dangerous Infections [in Russian], No. 1 (1975), p. 98.
6. V. P. Chernikov, V. I. Sorokovoi, and V. A. Shakhlamov, Byull. Éksp. Biol. Med., No. 8, 229 (1979).
7. N. B. Shalygina, "Functional morphology of the intestinal mucosa under normal conditions and in some acute intestinal infectious diseases," Author's Abstract of Doctoral Dissertation, Moscow (1978).
8. N. Dutta and M. Habbu, Br. J. Pharmacol., 100, 183 (1955).
9. T. S. Gaginella, J. C. Lewis, and S. F. Philips, Am. J. Dig. Dis., 22, 781 (1977).
10. T. Morishita, T. Hibi, H. Asakura, et al., Gastrointest. Endosc., 24, 284 (1978).
11. L. A. Turnberg, Clin. Sci. Mol. Med., 54, 337 (1978).

ACCUMULATION OF METHYLATED PURINES IN DNA OF RAT LIVER AND LARGE INTESTINE FOLLOWING REPEATED INJECTIONS OF 1,2-DIMETHYLHYDRAZINE

A. Ya. Likhachev and A. S. Petrov

UDC 615.277.4.015.42:[612.35+612.74].015.348

KEY WORDS: methylation of DNA; accumulation of O^6-methylguanine; intestinal tumors.

The compound 1,2-dimethylhydrazine (DMH) is widely used in experimental cancer research to induce carcinoma of the large intestine [3]. DMH exerts its carcinogenic effect evidently through interaction with DNA, by methylating its bases. After administration of a single dose of DMH to rats it has been shown that purine bases of DNA of various organs are methylated [1, 11, 12]. Meanwhile O^6-methylguanine, which is ascribed a leading role in malignant transformation of cells [7], is formed in fairly large quantities and preserved for a long time in the DNA of the large intestine, and also of the liver [12].

It was accordingly decided a matter of fundamental importance to study the changes taking place in the structure of DNA in the liver and large intestine of rats during long-term weekly administration of DMH. With this dosage, as is usually used in experimental research, malignant intestinal tumors arise comparatively quickly and in practically all animals. At the same time, tumors of the liver do not develop in such animals [3].

Professor N. N. Petrov Research Institute of Oncology, Ministry of Health of the USSR, Leningrad.

(Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 11, pp. 626-628, November, 1980. Original article submitted November 29, 1979.

0007-4888/80/9011-1619$07.50

EXPERIMENTAL METHOD

Ten noninbred male rats, divided into two groups, were used. The animals of group 1 (five rats) received a single subcutaneous injection of a neutral aqueous solution of tritiated DMH·2HCl (specific activity 3.2 mCi/mmole), prepared by the method in [2], in a dose of 0.48 mCi/kg. By the addition of unlabled DMH its dose was made up to 21 mg/kg calculated as the base. The animals of group 2 (five rats) received ^{3}H-DMH subcutaneously in the same dose, but as five injections, with weekly intervals between them. The rats of both groups were killed 12 h after the last injection by cervical dislocation and the mucous membrane of the large intestine was curetted with a scalpel. After curettage, two or three pieces of mucous membrane were taken from each rat for histological study. The remaining, lower part of the mucous membrane was separated from the underlying submucosa. Both parts of the mucous membrane, and also the liver were frozen in liquid nitrogen. DNA was extracted from the tissues by the phenolic method. The purine bases were fractionated chromatographically in a Sephadex G-10 column and their radioactivity determined on a Delta-300 scintillation counter (from Searle Analytic Inc., USA) by the method of Margison and Kleihues [8]. The concentration of methylated purine was expressed in micromoles per mole of the corresponding base, assuming that the specific activity of these alkylated products was half of the specific activity of the injected carcinogen [12].

EXPERIMENTAL RESULTS

The histological analysis showed that after curettage the fundal parts of the crypts and a very small quantity of subjacent connective tissue remained in the mucous membrane of the large intestine.

The maximal level of 7-methylguanine was discovered in the liver DNA (Fig. 1a). However, after a single injection of DMH its level in DNA of the liver and of both parts of the mucous membrane of the large intestine was lower than in animals receiving five injections of DMH. Under these circumstances, whereas the 7-methylguanine content in the liver DNA was increased by only 48%, in DNA from the lower part of the mucous membrane of the large intestine it was increased threefold.

After a single injection of DMH, O^6-methylguanine was detected in fairly low concentrations in liver DNA only, probably because of the low specific activity of the ^{3}H-DMH solution used. After five injections of the carcinogen the O^6-methylguanine level in the liver DNA was raised almost threefold. This product also was found in DNA of the lower part of the mucous membrane of the large intestine, but the amount of it there was one-third of that in the liver DNA. No O^6-methylguanine was found in the DNA of the upper part of the mucous membrane of the large intestine (Fig. 1b).

Besides 7-methylguanine and O^6-methylguanine, small quantities of methylated products of adenine were found in DNA of the organs studied from rats of both groups. For instance, 1-methyladenine, 3-methyladenine, and 7-methyladenine were found in the liver DNA, 3-methyladenine and 7-methyladenine in DNA from the lower part of the mucous membrane of the large intestine, and in DNA of the upper part of that organ 1-methyladenine was found, except in rats receiving five injections of DMH, when 3-methyladenine and 7-methyladenine were found. As a rule the level of these methyladenines was two or three times higher in the rats of group 2, which received five injections of DMH.

It will be clear from Fig. 2 that the level of radioactivity of adenine in DNA from different parts of the mucous membrane of the large intestine was considerably higher than in the liver DNA after a single injection of the carcinogen. After repeated injections of this compound, however, the radioactive label was incorporated into adenine of DNA of both parts of the mucous membrane of the large intestine more intensively than into adenine of liver DNA. Relatively smaller quantities of radioactivity were incorporated into guanine than into adenine, but the character of its accumulation in the DNA of the organs studied was similar to that of adenine.

Cumulation of 7-methylguanine and of certain methylated derivatives of adenine was thus observed in the DNA of all tissues tested. Comparatively large amounts of O^6-methylguanine were found in DNA of the lower part of the mucous membrane of the large intestine, an organ in which DMH selectively induces malignant tumors, after five injections of the compound. Meanwhile, no O^6-methylguanine whatever could be found in differentiating cells located in the upper parts of the crypts, whose DNA was studied separately. Data on the

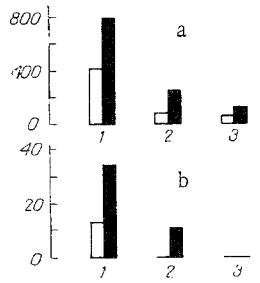

Fig. 1

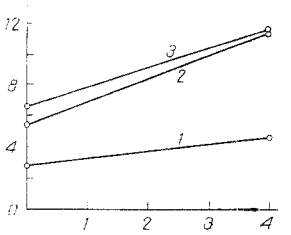

Fig. 2

Fig. 1. Methylation of guanine of DNA in different organs of rats after one (unshaded columns) or five (shaded columns) injections of ^{3}H-DMH. a) 7-Methylguanine (in moles % guanine × 10^4), b) O^6-methylguanine (in moles % guanine × 10^4). 1) Liver; 2) lower part of mucous membrane of large intestine; 3) upper part of mucous membrane of large intestine.

Fig. 2. Incorporation of radioactivity *de novo* into adenine of DNA in various rat organs after injections of ^{3}H-DMH. Abscissa, duration of exposure (in weeks); ordinate, radioactivity of adenine (in cpm/μmole adenine). Remainder of legend as to Fig. 1.

presence of O^6-methylguanine in DNA of enterocytes of the large intestine for 3 days [12], when these cells were renewed [3], and also the results of the present investigation are evidence that after administration of DMH, O^6-methylguanine was found in the DNA of cells which persist in the intestinal epithelium, i.e., in stem enterocytes located in the fundal regions of the crypts. Considering the leading role of stem cells in the genesis of malignant tumors of the large intestine [3], it can be concluded that the formation and long stay of the "promutagenic" base DNA-O^6-methylguanine [4] in the stem enterocytes, whose life cycle lasts only a few days [3], leads to the appearance of their transformed progeny.

A considerable rise in the level of O^6-methylguanine in the liver DNA after five injections of DMH is evidence that here also a cumulative effect may take place. This observation contradicts the results obtained in experiments with other carcinogens, in which cumulation of O^6-methylguanine in liver DNA did not take place [5, 8, 9]. This difference in the action of DMH on liver DNA can evidently be explained by the special character of metabolism of this carcinogen [3]. O^6-methylguanine is known to be formed in the DNA of various organs in the course of a few days after injection of DMH [11].

In these experiments O^6-methylguanine thus accumulated in liver DNA after repeated injections of DMH, i.e., a situation characteristic of a process of malignant growth was created, although no such growth took place. The cause of the resistance of liver tissue to the carcinogenic action of DMH, in our opinion, is most likely that this compound, unlike hepatotropic carcinogens, gives rise to injuries of the hepatocytes of a type which do not lead to their repair and to the replicative DNA synthesis associated with it — an essential factor for the initiation of carcinogenesis [10]. The appearance of liver tumors in rats with partial hepatectomy after administration of N-nitrosomethylurea, which normally does not induce neoplasms of the liver [6], confirms this suggestion.

LITERATURE CITED

1. A. Ya. Likhachev (A. J. Likhachev), G. P. Margison, and R. Montesano, Chem. Biol. Interact., 18, 235 (1977).
2. A. S. Petrov, A. Ya. Likhachev, and Yu. M. Kapustin, in: Carcinogenic N-Nitroso Compounds — Action, Formation, Determination (Proceedings of the 3rd Symposium) [in Russian], Tallin (1978), p. 163.
3. K. M. Pozharisski, A. Ya. Likhachev (A. J. Likhachev), V. F. Klimashevski, et al., Adv. Cancer Res., 30, 165 (1979).
4. P. J. Abbott and R. Saffhill, Br. J. Cancer, 36, 404 (1977).

5. H. K. Cooper, E. Hauenstein, G. F. Kolar, et al., Acta Neuropathol. (Berlin), 43, 105 (1978).
6. V. M. Craddock and J. V. Frei, Br. J. Cancer, 30, 503 (1974).
7. P. D. Lawley, Mutat. Res., 23, 285 (1974).
8. G. P. Margison and P. Kleihues, Biochem. J., 148, 521 (1975).
9. G. P. Margison, J. M. Margison, and R. Montesano, Biochem. J., 165, 463 (1977).
10. R. Montesano and H. Bartch, Mutat. Res., 32, 179 (1976).
11. K. J. Rogers and A. E. Pegg, Cancer Res., 37, 4082 (1977).
12. I. A. Swenberg, H. K. Cooper, I. Bucheler, et al., Cancer Res., 39, 465 (1979).