INHIBITION BY METHIONINE OF DEVELOPMENT OF MAMMARY GLAND CARCINOMA INDUCED BY 7,12-DIMETHYLBENZ(a)ANTHRACENE AND N-NITROSOMETHYLUREA IN RATS

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Epidemiological observations and experimental investigations have demonstrated the existence of the modifying influence of the calorific value and qualitative composition of the diet on the development of neoplasms. Obesity and excessive consumption of fat with the diet are regarded as factors of increased risk in the development of breast cancer in women [4, 5]. The addition of fat to the diet of laboratory rodents accelerates the appearance and increases the frequency of development of adenocarcinomas of the mammary gland in mice infected with Bittner's virus and induced by chemical carcinogens or by ionizing radiation in rats, whereas a deficiency of fat has an antitumor effect [5, 6]. However, keeping rats on a high fat diet, with a restricted level of lipotropic substances (choline, methionine, and folic acid) inhibits carcinogenesis in the mammary gland [13]. These observations have attracted attention to the role of lipotropic factors and, in particular, methionine, in the development of tumors. There is evidence that methionine is absolutely essential for growth of malignant cells *in vitro*, whereas normal cells can grow on media in which methionine is replaced by homocysteine [11]. On the other hand, methionine inhibited incorporation of ^{3}H-thymidine and ^{3}H-deoxyuridine into DNA of bone marrow cells [16] and inhibited carcinogenesis in the liver induced by ethionine, 2-acetylaminofluorene (AAF), and N-nitrosodiethylamine [9, 12, 14].

This paper gives data on the effect of an excess of methionine in the diet on the carcinogenic effect of 7,12-dimethylbenz(a)anthracene (DMBA) and N-nitrosomethylurea (NMU).

EXPERIMENTAL METHOD

Noninbred female albino rats aged 2 months were divided into two groups and kept on a standard diet with or without the addition of 0.5% methionine. Starting from the 5th day of the experiment, rats which received or did not receive methionine were given intravenous injections at weekly intervals of 1.5 mg DMBA (from Fluka, Busch, Switzerland; 3 doses) or 50 mg/kg NMU, synthesized in the Department of Organic Synthesis, Leningrad University (2 doses). The rats were palpated weekly to discover mammary gland tumors. Animals which died or which were killed 1 year after the beginning of the experiment were autopsied and any neoplasms discovered were studied microscopically. For statistical analysis of the data the chi-square test and P value method [15] were used.

EXPERIMENTAL RESULTS

As a result of the addition of methionine to the diet the frequency of development of adenocarcinomas ($P < 0.0001$) and fibroadenomas ($P = 0.025$) of the mammary gland induced in female rats by DMBA was considerably reduced (Table 1). Both the total number of these tumors (Fig. 1) and the multiplicity of their development (Table 1) were significantly reduced. In rats receiving and not receiving methionine with the diet the frequency of other neoplasms and the mean latent period of all tumors did not differ significantly. However, the length

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TABLE 1. Frequency and Localization of Tumors in Female Rats Treated with DMBA and NMU, with or without Methionine

Treatment	Number of rats		No. of tumors		Location and type of tumors									
					mammary gland				leukemia	Carcinoma of Tsymbal's gland		Mesenchymal tumors of the kidneys		Others
					adenocarcinoma		fibroadenoma							
	total	number with tumors	total	per rat	R	T	R	T		R	T	R	T	
DMBA	32	31 (97)	99	3,2	22 (69)	39	21 (66)	48	5 (16)	5	6	—	—	1a
DMBA + methionine	34	25 (74)	42	1,7	6 (18)*	7	14 (41)*	25	2 (6)	6	6	—	—	2b
NMU	28	26 (93)	55	2,1	9 (32)	10	5 (18)	5	9 (32)	1	1	15 (54)	22	8c
NMU + methionine	29	26 (90)	46	1,7	4 (14)*	5	6 (21)	8	5 (17)	1	1	17 (59)	23	4d

Legend. Percentages given in parentheses. R, T) Numbers of rats and tumors, respectively. a) Thecoma; b) osteosarcoma, uterine polyp; c) neuroma of optic nerve, ganglioneuroma, adenocarcinoma of the rectum, papilloma of the forestomach, adenoma of the adrenal cortex, two thecafolliculomas of the ovary and uterine polyp; d) thecoma, adenocarcinoma of the rectum, two adrenal neuromas.

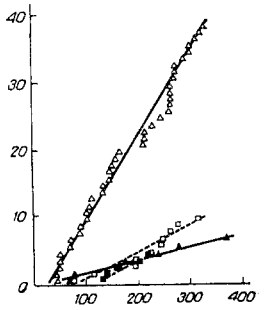

Fig. 1. Effect of methionine on development of adenocarcinomas of the mammary gland induced in rats by DMBA and NMU. Abscissa, latent period of tumors (in days); ordinate number of tumors. Empty triangles — DMBA, filled triangles — DMBA + methionine; empty squares — NMU, filled squares — NMU + methionine.

of survival of the animals was increased by methionine. For instance, toward the end of the experiment 41.2% of rats in this group were still alive, compared with only 15.6% in the group not receiving methionine ($P < 0.05$).

Keeping the rats on a diet with added methionine inhibited the development of adenocarcinomas of the mammary gland induced by NMU ($P = 0.05$), but had no significant effect on the frequency of neoplasms in other situations (Table 1) or the survival rate of the rats.

An excess of methionine in the diet thus inhibited the development of mammary gland tumors induced by DMBA and NMU but did not affect the appearance of neoplasms in other organs. Experiments showed that a diet with a low content of lipotropic agents stimulates induction of liver tumors by carcinogens of different classes, tumors of the large intestine by 1,2-dimethylhydrazine, and tumors of the esophagus by N-nitrosodimethylamine, but has no effect on carcinogenesis in the stomach induced by N-methyl-N'-nitro-N-nitrosoguanidine or the appearance of tumors of the esophagus, urinary bladder, and lungs following administration of N-nitrosodibutylamine [13, 14]. Data on the effect of a high fat diet with a low methionine content on activity of liver microsomal oxidases, which metabolize many carcinogens, and the

decrease in mutagenicity of the electrophilic compounds formed under these circumstances [13], allow some of the contradictions in the results of experiments using carcinogens with direct action and those requiring metabolic activation to be resolved to some extent. However, it is still not clear why when rats receiving AAF are kept on a diet with a low methionine content hepatocarcinogenesis is intensified but the development of mammary gland tumors is inhibited [13]. On the other hand an excess of methionine, which has antimutagenic [7] and anticytotoxic [3] activity, inhibits the development of liver and mammary gland tumors but does not affect induction of leukemias, tumors of the kidneys, or tumors of Tsymbal's gland [8, 9, 13, 14] (Table 1), and abolishes the inhibitory effect of hydroxyacetanilide on leukemia production [8]. These observations likewise do not agree with the view that the antitumor effect of an excess of methionine is due to its property of acting as the target for electrophilic forms of carcinogens [4, 12]. It must be pointed out, however, that when the diet is deficient in methionine [13] and when methionine is administered, the blood lipid level is lowered.

Administration of drugs with a hypolipidemic action (antidiabetic biguanides, clofibrate) gave the most marked antitumor effect against mammary gland tumors induced by carcinogens [1, 2]. Considering data on the hormonal dependence of mammary gland carcinoma [5, 6], on the one hand, and the positive correlation found between fat consumption and the blood levels of estrogens and prolactin [6], on the selective incorporation of ^{3}H-methionine into the neurosecretory cells of the hypothalamus [10], and on the role of S-adenosylmethionine in mediator metabolism in the brain, on the other hand, the possibility cannot be ruled out that the hypolipidemic and neurotropic effect of methionine may play an important role in its selective inhibitory effect on carcinogenesis in the mammary gland.

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