In the dot blotting test with serum against MMTV proteins, proteins with mol. wt. of 30,000, 36,000, and 60,000 daltons, which also gave cross reactions, were discovered in positively reacting specimens. Their investigation was made more difficult because of the tendency of the proteins to undergo degradation. Instability of the extracted proteins was perhaps connected with the use of preoperative radiotherapy (Fig. 2).

The results of the present investigation confirm the existence of antigens immunologically related to structural proteins of MMTV in HBT. Nucleotide sequences homologous with the sequences of MMTV have been found in the human genome, although no complete copy of the provirus has been found [4]. Positive results of searches for antigens similar to MMTV antigens in human material support the hypothesis [2] that the expression of these sequences is similar to that of endogenous mouse provirus. The results are in agreement with those obtained in [3], which demonstrated the considerable immunogenicity of tumors containing proteins resembling virus gp-52 for man compared with HBT, which do not contain it.

The results do not evidently reflect completely expression of the test antigens in all mammary gland tumors, for the tumor node proper did not always constitute the greater part of the preparation as received. Some HBT probably contain amounts of test antigens outside the limits of sensitivity of the methods used.

LITERATURE CITED

1. E. A. Komarova, I. N. Kryukova, and P. G. Komarov, Vopr. Virusol., No. 4, 461 (1980).
2. I. N. Kryukova, Éksp. Onkol., 2, No. 4, 3 (1980).
3. M. M. Black, R. E. Zachrau, and D. Shore, Bibl. Haematol., 43, 559 (1976).
4. R. Callahan, W. Drohan, S. Tronick, et al., Proc. Natl. Acad. Sci. USA, 79, 5503 (1982).
5. A. G. Farr and P. K. Nakane, J. Immunol. Methods, 47, 129 (1981).
6. D. Moore, I. Charney, and B. Kramarsky, Nature, 229, 611 (1971).

EFFECT OF METHYLCOBALAMINE ON METHOTREXATE UPTAKE BY NORMAL AND TUMOR TISSUES

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Methylcobalamine (MeCbl), a coenzyme of methionine synthetase (EC 2.1.1.13), a key component controlling the formation of reduced folates, stimulates proliferation of normal and tumor cells in vivo [1, 2]. An increase in the pool of proliferating cells in tumors with a low rate of proliferation, under the influence of MeCbl, enables the antitumor activity of cycle-dependent antimetabolites to be increased [2]. The therapeutic efficacy of the dihydrofolate reductase (EC 1.5.1.3) inhibitor, methotrexate (MTX), when used in combination with MeCbl, is increased as a result of inhibition of DNA synthesis in the greater part of the tumor cell population [3]. Meanwhile the effect of Cbl on MTX uptake by tumor cells cannot be ruled out. Cobalamines are known to facilitate uptake of methyltetrahydrofolic acid (the basic form of folic acid in the blood), which competes with MTX for cell membrane transport protein [5, 8], by hematopoietic cells. The problem of the effect of MeCbl on uptake of folate analogs, including MTX, by tumor cells has not previously been studied.

The aim of this investigation was to study the effect of MeCbl on [^{3}H]-MTX uptake by mammary gland adenocarcinoma cells and by the tissues of the small intestine and spleen of animals with transplanted tumors.

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TABLE 1. Changes in Kinetics of [^{3}H]MTX Uptake by Tissues after Administration of MeCbl

Tissue	Preparations injected	Dose of MeCbl, mg/kg	C_{max}, nanomoles/g ($\bar{X} \pm \tilde{x}$)	P (relative to control)	t_{max}, min	V_{in}, nanomoles/g/min ($\bar{X} \pm \tilde{x}$)	$t_{1/2}$, min
Tumor	MTX	—	5,3±2,0	—	30	0,28±0,40	63
	MeCbl and MTX	0,01	6,2±0,5	<0,01*	15	0,41±0,07**	69
		0,05	6,5±0,4	<0,01*	30	0,37±0,06**	57
		0,5	13,6±2,3	<0,01	60	0,70±0,19	45
Small intestine	MTX	—	15,9±4,0	—	30	0,72±0,21	75
	MeCbl and MTX	0,01	27,0±8,5	>0,05	30	1,75±0,54**	67
		0,05	32,3±9,0	<0,05*	30	1,42±0,42	63
		0,5	69,4±5,0	<0,001	30	3,48±0,35**	48

Legend. *) Differences significant by Fisher's F test, **) the same, at the $P < 0.05$ level.

EXPERIMENTAL METHOD

Mammary gland adenocarcinoma (Ca-755) was transplanted into female C57BL mice by subcutaneous injection of 10^6 cells. [^{3}H]-MTX (Amersham Corporation, England), with a specific activity of 580 GBq/mmole, was diluted with unlabeled MTX to 470 MBq/mmole, and a single dose of 10 mg/kg body weight was injected subcutaneously into the mice on the 6th day after transplantation of the tumor (control group). Animals of the other three groups received a single intramuscular injection of MeCbl (synthesized in the Laboratory of Chemistry and Technology of Thio-organic Compounds, "Vitaminy" Research-Production Combine), 15 min before injection of the MTX, in doses of 0.01, 0.05, and 0.5 mg/kg body weight. The animals were killed 15 and 30 min and 1, 2, 3, and 24 h after the injection of MTX (3-5 animals at each time).

The whole tumor, the spleen, and a segment of small intestine weighing 50-60 mg were isolated. The isolated tissues were homogenized and dissolved in 5% deoxycholic acid in 1 N NaOH for 24 h at 50°C. Radioactivity of the hydrolysates was measured on an Intertechnique SL-4221 scintillation counter in ZhS-8 liquid scintillator. The content of the preparation was expressed in nanomoles MTX/g wet weight of tissue. On the basis of the results a comparative analysis was undertaken of the kinetics of MTX uptake and elimination under the influence of MeCbl in the tumor, small intestine, and spleen of animals with adenoma Ca-755. The following parameters were used as criteria for assessment: maximal concentration of MTX (C_{max}) and the time taken to reach it (t_{max}) in the tumor and normal tissues, the rate of uptake of [^{3}H]-MTX during the first 15 min after injection of the preparation (V_{in}) and its half-elimination time from the tissues ($t_{1/2}$). The significance of differences between values for animals of the experimental and control groups was calculated by Student's and Fisher's tests [4].

EXPERIMENTAL RESULTS

The investigations enabled the effect of MeCbl to be estimated on [^{3}H]-MTX uptake into tumor, epithelial, and hematopoietic tissues of animals with Ca-755. Proliferating tissues are known to differ in their ability to take up MTX [7]. The results show that the maximal concentration of [^{3}H]-MTX in the tumor and small intestine increases with an increase in the dose of MeCbl given (Table 1). The [^{3}H]-MTX content in the spleen of these animals did not change significantly. Uptake of the compound into the target organs depends on the relative rates of its uptake and excretion. Calculation of these parameters shows that MeCbl stimulates uptake of MTX into the tumor and small intestine but does not affect the rate of its uptake by the spleen (Fig. 1). Meanwhile calculation of the half-elimination time of MTX from the tumor showed that with small doses of MeCbl the value of $t_{1/2}$ was virtually unchanged, whereas the rate of its elimination from the intestine and the half-elimination time of the preparation were less than in the control.

Acceleration of MTX uptake following a single injection with MeCbl resulted in the attainment of a higher intracellular concentration of exchangeable (not bound with dihydrofolate reductase) preparation in the tumor and small intestine. On inhibition of dihydrofolate reductase by MTX the pool of reduced folates in the cells is reduced, synthesis of thymidylate and purines de novo and also biosynthesis of amino acids are inhibited.

Meanwhile synthesis of purines de novo in the mucous membrane of the small intestine is known to be virtually nonexistent [6], and recovery of DNA synthesis after treatment with MTX takes place more rapidly than in the tumor [7]. For that reason, despite intensive accumulation of MTX in the small intestine, thanks to the use of ready-made purine nucleosides or purine nucleotide synthesis and to the more rapid recovery of thymidylate synthesis, the cytotoxicity of the preparation is weaker than in the tumor [6, 7].

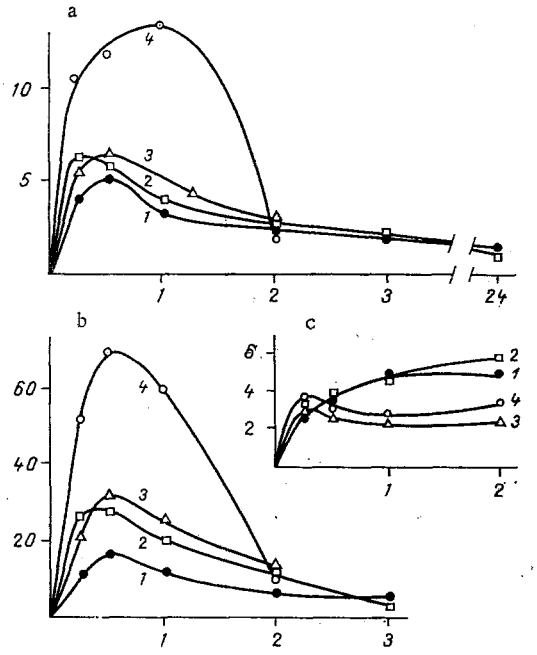

Fig. 1. [^{3}H]-MTX concentration in tumor (a), small intestine (b), and spleen (c) of mice with Ca-755 tumor. Abscissa, time after injection of [^{3}H]-MTX (in h); ordinate, MTX concentration (in nanomoles/g wet weight of tissue): 1) after injection of MTX; 2, 3, 4) after combined injection of MTX with MeCbl in doses of 0.01, 0.05, and 0.5 mg/kg respectively.

Analysis of the ratio between the rates of uptake and elimination of [^{3}H]-MTX in the tissues studied shows that a small dose of MeCbl (0.01 mg/kg) is suitable. In chemotherapeutic investigations, when a combination of this dose of MeCbl with MTX was used, inhibition of growth of the Ca-755 tumor was intensified and the life span of the animals lengthened [1].

It can thus be concluded from these results that the mechanism of the increased therapeutic efficacy of MTX under the influence of MeCbl is due not only to an increase in the number of DNA-synthesizing cells (which are those most sensitive to the inhibitory action of this antimetabolite, as was shown previously [2, 3]), in the tumor but also to more rapid uptake of the folate analog by the tumor cells.

LITERATURE CITED

1. F. G. Arsenyan, N. V. Myasishcheva, Z. P. Sof'ina, et al., Khim.-farm. Zh., No. 10, 49 (1978).
2. O. D. Golenko, F. G. Arsenyan, and N. V. Myasishcheva, Byull. Éksp. Biol. Med., No. 2, 185 (1980).
3. N. V. Myasishcheva, Z. P. Sof'ina, O. D. Golenko, et al., Éksp. Onkol., No. 5, 29 (1982).
4. V. Yu. Urbakh, Statistical Analysis in Biological and Medical Research [in Russian], Moscow (1975).
5. J. D. Goldman, Ann. N. Y. Acad. Sci., 186, 400 (1971).
6. R. C. Jackson and B. J. Harkrader, Nucleosides and Cancer Treatment, Sydney (1981).
7. F. M. Sirotnak, Cancer Treat. Rep., 65, Suppl. 1, 19 (1981).
8. G. Tisman and V. Herbert, Blood, 41, 465 (1973).