The results of the present experiments show that the stimulating action of TP on induction of renal capsule angiosarcomas by DMH is due to the effect of the hormone on the tumor initiation phase, in the complete absence (at least under these experimental conditions) of any promotor effect. Bakshi et al. [6] showed in an Ames' system with dimethylnitrosamine that the microsomal fraction from male kidneys gave a much greater mutagenic effect than that from female kidneys, and that this effect was potentiated even more by administration of an androgen. Another theoretically possible mechanism of the stimulating action of TP is through β-glucuronidase. It is considered [1] that conjugates of DMH metabolites with glucuronic acid, formed in the liver, are broken down in the target organ by the action of β-glucuronidase, whose activity in the kidneys is induced by androgens [8].

Sarcomas of the kidney capsule, including those of vascular genesis, are also known in man; they are found equally infrequently in men and women, and like the experimental tumors, they can infiltrate kidney tissue or leave it intact [7].

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BIOCHEMICAL DIFFERENCES IN TUMOR CELLS OF EHRLICH'S ASCITES CARCINOMA STRAINS SENSITIVE AND RESISTANT TO 5-FLUOROURACIL

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KEY WORDS: 5-fluorouracil; RNA; adenylate cyclase system; Ehrlich's ascites carcinoma.

In the search for biochemical criteria of sensitivity and resistance of tumor cells to 5-fluorouracil (5FU) the principles governing accumulation of 5FU in the acid-soluble fraction (ASF) and its incorporation into RNA were studied in experiments *in vitro* and *in vivo* and the state of the adenylate cyclase system was compared in strains of Ehrlich's ascites carcinoma sensitive and resistant to 5FU.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino mice weighing 20-25 g into which an Ehrlich's ascites carcinoma naturally sensitive to 5FU was transplanted. A strain of the tumor with induced resistance to 5FU was obtained in mice into which, after transplantation of the tumor, 5FU was injected intraperitoneally daily in a dose of 15 mg/kg body weight. The strain of tumor resistant to 5FU was obtained at the 20th subculture of the tumor.

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TABLE 1. Accumulation of [6-^{3}H]-5FU in ASF and Its Incorporation into RNA (n-moles/min/mg DNA) from Tumor Cells of Strains of Ehrlich's Ascites Carcinoma Sensitive and Resistant to the Preparation

Strain of tumor	ASF		RNA	
	incubation time, min			
	30	60	30	60
Sensitive	0,358±0,004	0,662±0,07	0,045±0,005	0,072±0,008
Resistant	0,141±0,015	0,154±0,02	0,017±0,002	0,019±0,002

TABLE 2. Accumulation of [6-^{3}H]-5FU in ASF (in moles/min/mg DNA) and Incorporation into RNA of Tumor Cells of Strains of Ehrlich's Carcinoma Sensitive and Resistant to 5FU (in moles/min/mg RNA), and into Cells of the Liver, Spleen, Kidneys, and Mucosa of the Small Intestine of Mice with Tumors during Incubation for 60 min

Test object	ASF		RNA	
	I	II	I	II
Tumor cells	132,65±14,66	79,35±3,21	85,56±7,97	36,77±2,75
Liver	339,10±41,44	311,12±29,69	12,88±1,32	11,25±0,87
Spleen	112,77±12,56	96,11±8,35	2,76±0,33	2,35±0,11
Kidneys	248,36±25,65	210,52±11,39	3,44±0,41	3,56±0,17
Mucous membrane of small intestine	86,68±8,56	75,57±2,72	9,62±0,73	8,76±0,56

Legend. I) Tumor cells and organs of animals with strain of tumor sensitive to 5FU, II) with strain resistant to 5FU.

In experiments *in vitro* a 10% suspension of cells in Eagle's medium was prepared from tumor cells. [6-^{3}H]-5FU (specific activity 104-192 GBq/mmole, Czechoslovakia) was added in a dose of 37 kBq to individual samples and the intensity of accumulation of the preparation in the ASF and of its incorporation into RNA were determined after incubation for 30 and 60 min. After incubation the tumor cells were washed and homogenized in the cold in 0.5 N $HClO_4$ solution. The ASF and lipids were extracted from the homogenates and DNA was separated from RNA [4]. The radioactivity of the samples was determined on a Mark III scintillation counter (Nuclear Chicago, USA).

In the experiments *in vivo* the labeled preparation was injected into mice with sensitive and resistant strains of the tumor intraperitoneally in a dose of 1850 kBq per mouse. The mice were killed 60 min later, tumor cells and also the liver, spleen, kidney, and mucous membrane of the small intestine were removed, and accumulation of the preparation in the ASF and its incorporation into RNA were studied in homogenates.

Activity of adenylate cyclase [5] and AMP phosphodiesterase [1] was studied in homogenates of the tumor cells and their concentration of cAMP and cGMP was determined by radioimmunoassay with kits from Amersham Corporation (England) [3]. Protein in the homogenates was determined by Lowry's method [2].

EXPERIMENTAL RESULTS

Significant differences in the accumulation of [6-^{3}H]-5FU in the ASF and its incorporation into RNA of the tumor cells were found between strains of Ehrlich's ascites carcinoma sensitive and resistant to 5FU (Table 1). Accumulation of the preparation in ASF of tumor cells of the sensitive strain was twice as high as in the tumor strain resistant to the drug during the first 30 min of incubation. During the next 30 min of incubation accumulation of 5FU in the ASF of the sensitive strain of the tumor was doubled, whereas in tumor cells of the resistant strain it was increased only very little. The same pattern was also found for incorporation of [6-^{3}H]-5FU into RNA of tumor cells belonging to strains sensitive and resistant to the compound; however, incorporation of the compound into RNA was an order of magnitude lower than its accumulation in the ASF.

In experiments *in vivo* (Table 2) very high accumulation of [6-^{3}H]-5FU in the ASF of tumor cells sensitive to the compound was observed, but only half as much of it accumulated in the

TABLE 3

Parameter tested	Sensitive strain	Resistant strain	P
Activity, nmoles/mg protein/min			
adenylate cyclase	1,72±0,17	3,88±0,26	0,05
phosphodiesterase	360±36,3	203±22,4	0,05
Concentration, pmoles/mg protein:			
cAMP	2,54±0,22	7,63±0,26	0,001
cGMP	0,399±0,088	0,150±0,028	0,05

ASF of tumor cells resistant to the compound. There was also considerable accumulation of 5FU in ASF from the liver, kidneys, spleen, and intestinal mucosa of animals with tumors of strains sensitive and resistant to the compound; differences in accumulation of 5FU in ASF from organs of mice with different strains of the tumor were not significant.

Very high incorporation of [6-^{3}H]-5FU was found in RNA of tumor cells of the strain resistant to the compound, which was 85 nmoles/min/mg protein, whereas its incorporation into RNA of tumor cells resistant to the compound was about half this value. Incorporation of 5FU into RNA of the liver, spleen, kidneys, and mucosa of the small intestine was comparatively low and amounted to 2-10% of its accumulation in the ASF of the corresponding organs, further confirmation of the selective action of 5FU on tumor cells.

The results of determination of adenylate cyclase and cAMP phosphodiesterase activity and of the concentrations of cyclic nucleotides in tumor cells of strains of Ehrlich's ascites carcinoma sensitive and resistant to 5FU are given in Table 3.

They show that adenylate cyclase activity in tumor cells of the sensitive strain was lower, whereas cAMP phosphodiesterase activity was higher than activity of the corresponding enzymes in tumor cells of the resistant strain. As a result of this relationship, the enzyme activities and cAMP concentration were about three times higher in tumor cells of the resistant strain than in those of the sensitive strain. All differences found in enzyme activity and cyclic nucleotide concentration were significant.

If the cAMP level in the tumor cells was low, 5FU gave a good therapeutic effect, but if the cAMP level was high, the proliferative pool of tumor cells was reduced and the number of cells in the stationary phase increased, and as a result, therapeutic activity of 5FU was low in the treatment of such tumors.

The significant differences found in accumulation of labeled 5FU into the ASF and in incorporation of the compound into RNA of tumor cells differing in their sensitivity to 5FU, and also the appreciable differences in adenylate cyclase and phosphodiesterase activity and in the cyclic nucleotide concentrations in tumor cells differing in their sensitivity to the compound can be used as reliable criteria for determination of individual sensitivity of tumors to the action of 5FU.

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