brain and skin tissue was revealed for the first time during the latent period after injection of neurinoma cells. It can be tentatively suggested that after removal of the activator and a reduction in activity of the enzyme, favorable conditions were created for growth of the graft. In fact, on the 8th-9th day the tumor nodule in the brain tissue could be detected morphologically and visually. The increase in arginase activity on the 16th day in the neoplasm was an independent phenomenon, for no change in arginase activity could be found in other parts of the brain or skin. This fact can evidently be explained by the characteristics of metabolism of this tumor. A similar change in arginine activity has been observed in hepatomas induced by azo compounds, and associated with the need for rapidly growing tissues to synthesize additional polyamines [7]. The possibility cannot be ruled out that the transient increase in arginase activity in the neurinoma was due to the same cause. The results of these experiments, indicating sporadic changes in arginase activity during the period of growth of the neurinoma, are in agreement with the concept of nonuniformity of tumor growth.

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DIFFERENCES IN MICROSOMAL MONO-OXYGENASE ACTIVITY IN CELLS OF ASCITES AND SOLID TUMORS

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A serious obstacle to the detailed study of microsomal mono-oxygenases (MMO) in tumor cells, especially during long-term passage, is their extremely low activity. Nevertheless, this is a problem of great practical importance, because MMO metabolize not only important endogenous substrates (hormones, cholesterol), but also most hydrophobic xenobiotics, for they activate and detoxicate many antitumor agents. The most important aspect of the problem is the study of factors determining MMO activity. In normal cells activity of this very important system of adaptive enzymes is determined not only by the character of the substrates to be metabolized (inducers, inhibitors), and by sex, age, linear, and tissue differences [3, 12], but also by the position of the cell in the complex structure of the organ [2].

The aim of this investigation was to determine how activity of aryl hydroxylase (AH), one of the most important characteristics of MMO, depends on the type of organization of the tumor cells. In particular, activity and inducibility of AH were compared in ascites and solid forms of three transplantable tumors.

EXPERIMENTAL METHOD

Male mice weighing 23-25 g were used, eight to 10 animals in each experimental group. Sarcoma MCh-11 [8] was transplanted into C57BL/6j mice, hepatoma 22a [15] into C3HS mice, and Ehrlich's tumor (from the

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TABLE 1. AH Activity (in picomoles 3-OH/BP/min/mg protein) in Tumors (M±m)

Tumor	Solid		Ascites		In culture	
	control	expt. (3-MCh)	control	expt. (3-MCh)	control	expt. (3-MCh)
Hepatoma 22a	1,33±0,20	1,87±0,3	0,04±0,02	0,17±0,03	5,7±1,5	10,3±2,4
Ehrlich's tumor	0,80±0,20	2,1±0,5	0	0	—	—
Sarcoma MCh-11	0,58±0,10	1,22±0,10	0,05±0,03	0,10±0,04	—	—

TABLE 2. Effect of Inhibitors on AH Activity in Tumors (M±m)

Tumor	Residual activity, %			
	during action of 7,8-benzoflavone		during action of metyrapone	
	control	expt. (3-MCh)	control	espt. (3-MCh)
Hepatoma 22a in culture	12,6±4,5	7,4±2,4	98,5±7,0	113±17
Ascites	—	12,5±2,0	—	81±12
In culture	35,6±6,0	11,6±3,0	101±4	92,6±12,0
Sarcoma MCh-11	39±6	24±5	102±8	—

Laboratory of Tumor Strains, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR) into noninbred mice. Microsomes were isolated from solid tumors 12-14 days after subcutaneous injection of 10^6 ascites cells, when the tumor weighed 1.5-2 g. The ascites form of tumor was studied on the 7th day after intraperitoneal injection of 10^6 ascites cells. As inducer of the MMO system 3-methylcholanthrene (3-MCh, from Koch Light, England) in olive oil was injected in a dose of 50 mg/kg for 2 days before the experiment. In the case of solid tumor growth the inducer was injected intraperitoneally, and in the case of ascites growth – subcutaneously. A culture of hepatoma 22a was grown in Eagle's medium with 50% lactalbumin hydrolysate and 20% serum. 3-MCh was added to the culture medium 48 h before isolation of the microsomes up to a concentration of 0.2 μg/ml. The cells were detached with trypsin. Microsomes were isolated at 4°C in a solution of 1.15% KCl, 0.05 M Kh_2PO_4, 1 mM EDTA, 1 mM dithiothreitol, and 0.1% trasylol, pH 7.4. During isolation of microsomes from solid tumors a 20% tissue homogenate was obtained by means of a Potter's mechanical homogenizer. Ascites cells and cells from a culture of hepatoma 22a were washed three times in isolation medium and disintegrated on an MSE ultrasonic homogenizer (500 W). Ultrasonic treatment of homogenates of solid tumors did not affect MMO activity. The resulting tissue homogenates were centrifuged at 9000 g for 20 min, then at 105,000 g for 1 h. The residue was resuspended in 0.1 M $Na_4P_2O_7$ and 1 mM EDTA, pH 7.4, and recentrifuged for 1 h at 105,000 g. AH activity was determined by measuring 3-OH-benzpyrene (BP) [1, 9] on a Perkin Elmer spectrofluorometer. The incubation time was 45 min at 37°C and the concentration of microsomal protein 1.5-2 mg/ml. The calibration curve was plotted over the range from 5 to 500 pM 3-OH-BP. To determine the effect of inhibitors, 7,8-benzoflavone was added to a concentration of 10^{-5} M in the incubation medium, and metyrapone to a concentration of 10^{-4} M. Protein was determined by Lowry's method in the modification in [5].

EXPERIMENTAL RESULTS

The presence of functionally active NADPH-dependent AH was demonstrated in these experiments in microsomes isolated from solid forms of tumors (Table 1). The basal level of activity in the tumors studied was low, in agreement with data in the literature [1, 2, 10, 12], and was closely similar, even though in the original tissues this parameters differs by more than one order of magnitude: liver > mammary gland > vascular endothelium [3]. Convergence of this kind may be due not only to known factors (malignant change, selection, progression), but also to the fact that cells, which existed previously in an organ with a strictly definite structure, find a more uniform organization when they are present in the tumor.

A change in organization of the tumor cells (growth in the ascites form) led to a marked and parallel change in MMO activity (Table 1). The quantity of 3-OH-BP formed from BP by microsomes of hepatoma 22a and sarcoma MCh-11 cells was 30 and 11 times less respectively than in the solid tumors, and in the case of Ehrlich's tumor none whatever was found. It is difficult as yet to judge the universality of this phenomenon because of the absence of comparative studies of this kind in the literature. The only reference found is to an

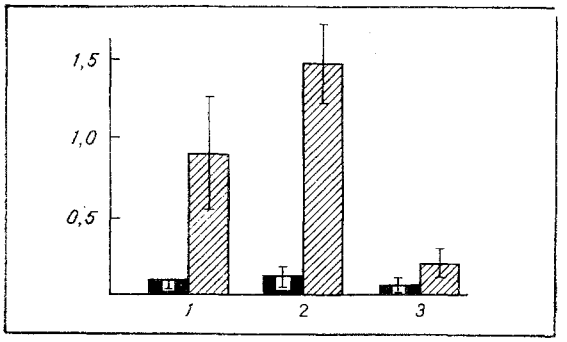

Fig. 1. Dependence of AH activity on duration of growth of ascites hepatoma 22a. Ordinate, enzyme activity (in picomoles 3-OH-BP/min/mg protein). Black columns indicate basal level of AH activity in tumor, obliquely shaded columns – level of AH activity in tumor after induction by 3-MCh. 1) 3rd day, 2) 5th day, 3) 7th day of growth of tumor.

extremely low cytochrome P-450 level in cells of Yoshida's ascites hepatoma [10], but this was not compared with the corresponding solid form.

The AH level in ascites and solid forms of sarcoma MCh-11 was raised equally. In Ehrlich's solid tumor it was increased by 2.6 times, but in the ascites form no 3-OH-BP could be found even after the action of the inducer. Incidentally, hepatoma 22a cells, adapted to growth in culture, had a higher level of basal and induced MMO activity than cells of the original tumor (Table 1). This could be the result not only of a change in the form of organization of the cells, but also of their selection as a result of long-term culture in vitro. The importance of this last factor when ascites and solid forms are compared is less than in the case with a culture of hepatoma 22a, for solid tumors were obtained in every experiment by subcutaneous injection of ascites cells. Under these circumstances the rate of growth and activity of MMO did not differ in tumors obtained by solid– solid and ascites– solid transplantation.

To compare the qualitative composition of MMO in tumors, inhibitor analysis was undertaken. Two AH isozymes, differing in the action of 7,8-benzoflavone on them in vitro, are known to exist in normal tissues: activity of one isozyme is potentiated, whereas that of the other is inhibited, by 7,8-benzoflavone [3]. In the present experiments 7,8-benzoflavone inhibited both basal and induced MMO activity (Table 2).

The other inhibitor of the MMO system, metyrapone, was ineffective. The isoform cytochrome P_1-450 was thus found in both connective-tissue and epithelial tumors. The results agree with those obtained by other workers [12], who showed that cytochrome P_1-450 is the predominant isoform in mouse tumor cells. This fact is evidence of convergence of the properties of MMO in tumor cells in the same direction.

Although analysis of individual factors influencing MMO activity in ascites and solid tumors is difficult because of their complexity and interdependence, they can be conventionally subdivided into "humoral" and "geometric." We know, for example [6], that cells of one of the L sublines (LSF), adapted to growth on serum-free medium, are more capable of oxidizing BP under these conditions that the original L cells or LSF cells on medium with serum. The possibility cannot be ruled out that cells existing in the depth of the tumor nodule are partly adapted to existence under conditions of restricted access to nutrients and oversaturation with metabolic products by a compensatory rise of MMO activity. Ascites tumor cells, at least in the logarithmic phase of growth, are under more optical conditions and their MMO activity may be lower.

Since the ascites tumor is more homogeneous in the degree of proliferative activity of the system than the solid tumor [11], it was decided to study dependence of AH activity on the time of development of the tumore. It was found that the basal level of AH activity on the 3rd or 5th days of growth of heptama 22a is higher than on the 7th day, but much lower than in the solid tumor (Fig. 1). However, after induction by 3-MCh, MMO activity in tumor microsomes, isolated on the 3rd and 5th days of growth of the tumor, was increased and was equal to the basal level of activity in the solid tumor. These data are interesting in connection with the results of experiments on cell lines [7]. These cells, with a low basal AH level, were most sensitive to

induction in the S-phase of the cell cycle. The greater inducibility of the MMO system of ascites hepatoma 22a cells on the 3rd and 5th day of tumor growth to perhaps connected also when the larger number of cells in the S-phase, which are more sensitive to the action of the inducer. Not only reduction of the proliferative pool, but also a shift in cell composition, may play a role in the depression of induction activity on the 7th day of growth, as is the case of Ehrlich's IFKh tumor, in which the ratio between the two original subclones changes with growth of the tumor [4]. As regards the role of "geometric" factors, it has been shown on primary liver cultures, by changing the adhesiveness of the substrate, that AH activity and the cytochrome P-450 concentration depend on the degree of spreading of the cells. Maximal activity of MMO enzymes was observed in hepatocytes cultured on highly adhesive substrates [13]. Furthermore, differences connected with the influence of surrounding tissues are found for ascites and solid cells. We know [14] that the concentration of cytochrome P-450 is higher in Morris hepatomas 7795 and 5123D, transplanted into the liver, than in the same tumors growing intramuscularly.

On the whole the results of this investigation are evidence that MMO activity in tumor cells may be determined, as well as by other factors, by the form of their organization.

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