tients with a longer period of survival [5, 8, 14]; the state of the B-dependent zones and the discovery of sinus histiocytosis have been interpreted differently [5, 8, 10, 12]; weak activity or exhaustion of LN is regarded as a bad prognostic sign [8, 10]. The results confirm existing views on the connection between metastasization and activity of regional LN. Meanwhile absence of a response of the remote LN will be noted.

The results are evidence that growth of HAPC in mice is accompanied not only by a leukemoid response of myeloid type [4], but also by atrophy of the thymus, activation of T- and B-dependent zones in the regional ipsilateral and contralateral LN, with preservation of the usual structure of distant LN (mesenteric LN and Peyer's patch). Activation of LN may be connected with the immunologic response to the tumor or it may be a compensatory reaction to the developing lymphocytopenia in the blood and gradually increasing atrophy of the thymus.

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EFFECT OF GANGLIOSIDES SECRETED BY ASCITES HEPATOMA 22a CELLS ON INTENSITY OF PROTEIN SYNTHESIS IN THESE CELLS AND ON THEIR SENSITIVITY TO INDOCARB

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The important biological role of gangliosides is largely determined by their location on the cell surface. Cell recognition and cell adhesion, and the ability of normal cells to exhibit contact inhibition and the loss of this property during malignant transformation all depend on the ganglioside composition of membranes. The membrane receptor of Vibrio cholerae is a ganglioside; gangliosides of the cell surface participate in reception of other bacterial toxins, viruses, interferon, and peptide hormones [4]. There is evidence of the role of gangliosides in serotonin reception [2, 12].

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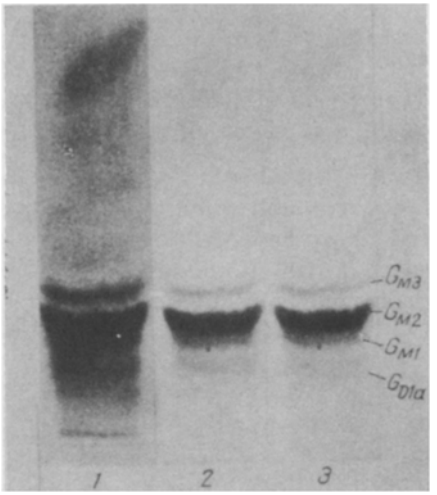

Fig. 1. Thin-layer chromatography of gangliosides. 1) Ascites hepatoma 22a cells; 2) ascites fluid; 3) medium conditioned by hepatoma cells.

TABLE 1. Effect of Gangliosides of Hepatoma on Protein Synthesis in Hepatoma 22a Cells

Ganglioside	Concentration, μM	Protein synthesis, %*	
		40 min	60 min
G_{M2}	0,01—0,04	127	205
	0,07—0,1	150	217
G_{D1a}	0,05—0,08	129	211
	0,1—0,5	160	232

Legend. *) Incorporation of [^{14}C]leucine into protein in absence of gangliosides taken as 100%; cell concentration 3×10^5/ml; mean results of five experiments shown, error ±10%.

Gangliosides can take part in intracellular regulatory functions of serotonin and other biogenic monoamines [1, 12]. Data indicating the existence of these functions have been obtained on embryonic and tumor cells [8, 10]. It has been shown, in particular, that many structural analogs of biogenic monoamines which act at the intracellular level specifically inhibit cell division and protein biosynthesis [1, 17]. Experiments on early sea urchin embryos have shown that these effects grow weaker or disappear completely if gangliosides are added to the medium [8]. It therefore seemed important to carry out similar experiments on tumor cells, more especially because that would allow the corresponding effects to be tested not only for exogenous gangliosides, but also for gangliosides secreted into the medium by the cells themselves.

In the investigation described below the effect of gangliosides secreted into the medium by cells of mouse ascites hepatoma 22a on the sensitivity of these cells to indocarb, a cytotoxic analog of serotonin, was investigated. This substance was chosen as a highly active serotoninolytic [5] which inhibits protein biosynthesis in embryonic and tumor cells [7, 8]. The effect of gangliosides on the intensity of protein synthesis also was studied, for sensitivity of the cells to indocarb was judged by the changes in this parameter.

EXPERIMENTAL METHOD

Tumor cells were isolated from the peritoneal cavity of male (CBA × C57BL/6) F_1 mice on the 9th day after transplantation, washed three times with physiological saline, and resuspended in Eagle's medium without leucine, diluted with physiological saline (1:1 or 1:2). The intensity of protein biosynthesis was determined by the standard method based on incorporation of [^{14}C]leucine (Amersham Corporation, England; specif-

TABLE 2. Effect of Gangliosides of Hepatoma on Sensitivity of Hepatoma 22a Cells

Experimental conditions	Concentration, μM	Inhibition of protein synthesis, % of control
Indocarb	13	43*
Indocarb + G_{M2}	13+(0,01—0,04)	21**
Indocarb + G_{M2}	13+(0,07—0,1)	20**
Indocarb + G_{D1a}	13+(0,05—0,08)	17**

Legend. *) Incorporation of [^{14}C]leucine into proteins in Eagle's medium taken as 100%, **) control incorporation of [^{14}C]leucine into proteins determined in presence of same concentrations of gangliosides; cell concentration 3×10^5/ml; mean results of six experiments shown; error ± 10%.

ic activity 330 mCi/mmole, activity in incubation medium 0.08-0.1 μCi/ml) in the protein fraction insoluble in hot TCA. The suspensions (1×10^8 or 3×10^5 cells in 1 ml) were incubated at 37°C with continuous shaking for 40-60 min in the presence of the test ganglioside fractions; indocarb was added, followed 20 min later by [^{14}C]leucine, and the mixture was then incubated for a further 20 min. Sometimes instead of gangliosides, incubation medium conditioned by cells was used. To obtain it, the donor's cells (3×10^7 in 1 ml) were incubated in the usual way for 60 min, after which the cells were removed by centrifugation and the recipient's cells (3×10^5 in 1 ml) were resuspended in the resulting conditioned medium.

The gangliosides were studied as follows. Native ascites hepatoma (1×10^8 cells in 1 ml) was centrifuged (30 min, 5000 rpm) and the sedimented cells were washed three times, resuspended in Eagle's complete medium (1×10^8 or 1×10^5 cells/ml) and incubated for 45-60 min at 37°C, after which they were sedimented by centrifugation and used for isolation of gangliosides [9]. The ascites fluid and conditioned medium after removal of the cells were lyophilized and the gangliosides were extracted and isolated [9]. Analytical chromatography of the gangliosides was carried out on 6 × 6 cm plates with KSK silica-gel (5-7 μ) in a chloroform–methanol–2.5 N ammonia (60:35:8) system; the chromatograms were developed with resorcin reagent [11]. Preparative isolation of gangliosides G_{M2} and G_{D1a} from the hepatoma cells was carried out as described previously [6]. The sialic acid content was determined as in [11].

EXPERIMENTAL RESULTS

In agreement with data obtained previously, the hepatoma 22a cells contained two principal gangliosides – G_{M2} and G_{D1a} (57 and 17% respectively) – and also a number of minor components [6]. The total ganglioside content was 4 μg per 1×10^8 cells. The same principal types of gangliosides were present in the ascites fluid and in medium conditioned by cells (1×10^8 cells in 1 ml; Fig. 1). The ganglioside content was 0.7 μg/ml in ascites fluid and 0.4 μg/ml in the conditioned medium.

Dilution of the native hepatoma cell suspension (1×10^8 cells/ml) 300-fold had little effect on the relative intensity of protein synthesis in them, whereas addition of gangliosides to the incubation medium in concentrations comparable with their concentrations in ascites fluid or even in lower concentrations stimulated protein biosynthesis appreciably in a liquid cell suspension (Table 1). Indocarb, in a concentration of 4-6 μg/ml (10-16 μM), inhibited protein biosynthesis in the diluted cell suspension (3×10^5 cells/ml; Table 2). In the case of the native suspension (1×10^8 cells/ml) hepatoma cells were in general insensitive to indocarb in this concentration.

The inhibitory action of indocarb was completely or partly prevented in cases when the "diluted" hepatoma cells (3×10^5/ml) were resuspended in conditioned incubation medium. Gangliosides G_{M2} and G_{D1a}, taken in concentrations close to those in the incubation medium, also had a better protective action against indocarb.

Ascites hepatoma 22a cells, taken in the native concentration (1×10^8/ml), thus secrete gangliosides G_{M2} and G_{D1a} into the incubation medium in sufficient quantities to make these cells insensitive to the action

of indocarb, a structural analog of serotonin. When the cell concentration in the medium is 3×10^5/ml the concentration of gangliosides secreted by them is insufficient to exhibit their protective action and the cells remain sensitive to indocarb. Their sensitivity weakens or disappears completely if the cells are suspended in incubation medium, i.e., in medium with a sufficiently high ganglioside concentration or in the presence of exogenous gangliosides.

The protective action of gangliosides against indocarb is evidently not the simple result of the ability of the gangliosides to stimulate protein biosynthesis in hepatoma cells. To judge from the results of experiments with resuspension of cells in conditioned medium, a protective action against indocarb was observed not only in the complete absence of stimulation of protein synthesis, but also when synthesis was appreciably inhibited.

The mechanism of the protective and stimulating action of gangliosides secreted into the medium by hepatoma 22a cells is not yet clear. We know that electrostatic interaction takes place in solutions between gangliosides, on the one hand, and serotonin and its structural analogs on the other hand [3]. However, the protective effect now demonstrated cannot be explained by simple binding of indocarb with gangliosides in the medium, for the concentration of indocarb used was two orders of magnitude higher than the protective concentration of gangliosides. Gangliosides of ascites fluid or of the conditioned medium evidently affect surface and (or) intracellular receptors of the cells. Gangliosides of the medium are evidently in a state of equilibrium exchange with gangliosides of the cytoplasmic membranes. It can be tentatively suggested that disturbance of this equilibrium taking place, in particular, on dilution of the cell suspension, leads to changes in the receptor properties of the cells. Exchange of gangliosides between cells and surrounding medium can be regarded in this case as a natural regulatory mechanism.

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