The new colchicine-resistant mouse cell line B-82$^{CH^R}$-9 described above may prove a convenient model not only for the study of the biochemical and genetic mechanisms of changes in plasma membrane permeability but also in cell hybridization experiments, for the fact that it contains two selective markers (TK$^-$, colchicine-resistance) may allow hybrid cells to be selected during fusion with cells that have no selective markers.

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EFFECT OF CERTAIN DRUGS ON SURVIVAL OF CHINESE HAMSTER FIBROBLASTS in vitro

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Mediators of the nervous system (acetylcholine, serotonin, catecholamines) of some invertebrates and of lower vertebrates are known to be present a long time before formation of the nervous system [2, 3]. For instance, in the organism which has been studied most extensively in this respect, namely the early sea urchin embryo, a serotonin-like substance, dopamine, and acetylcholine have been found in concentrations which vary in the course of development [1, 4, 7]. Neuropharmacological preparations which are antagonists of these "prenervous" mediators induce blockade of cleavage division and protein synthesis in developing sea urchin and molluscan embryos, in connection with inhibition of mediator functions [2].

Like embryonic cells, cells of ascites tumors (Ehrlich's carcinoma and hepatoma 22a) also are sensitive to certain neuropharmacological agents with the property of antagonists of serotonin and catecholamines. These substances cause inhibition or blockade of protein synthesis [8]. Similar results have recently been obtained on mammalian cells cultured in vitro [5, 10]. For instance, the work of Vernadikis et al. has shown that BAS (1-benzyl-2,5-dimethylserotonin hydrochloride) and antihistamine preparation No. 202 inhibit incorporation of ^{14}C-lysine into the TCA-insoluble protein fraction of Chinese hamster fibroblasts.

In the investigation described below an attempt was made to determine whether antiserotonin and antihistamine drugs, and also serotonin and histamine themselves, influence fibroblast growth in culture, and also whether serotonin or a substance resembling serotonin is present in these cells.

EXPERIMENTAL METHOD

Experiments were carried out on a continuous line of Chinese hamster fibroblasts strain B11 dii FAF-28, clone 431. The cultures were grown at 37°C on medium consisting of equal parts of Eagle's medium and

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TABLE 1. Effect of Bicarphen and Histamine on Survival of Chinese Hamster Fibroblasts in Culture (in % of control)

Substance	Concentration, μg/ml	Survival rate, %
Bicarphen	5	24,9
Histamine	5	73,4
	7	70,5
	9	71,9
Bicarphen + histamine	5+5	72,5
	5+7	76,2
	5+9	72,5

TABLE 2. Effect of Preparation No. 407 and Serotonin and Survival of Chinese Hamster Fibroblasts in Culture (in % of control)

Substance	Concn., μg/ml	Survival rate, %
Preparation No. 407	5	83,4
	10	78,5
	20	56,0
Serotonin	5	83,7
	10	98,1
	20	98,2
Preparation No. 407 + serotonin	20+20	45,4
	10+10	67,8
	20+10	36,7
	5+5	67,2

medium No. 199, 20% inactivated bovine serum, and antibiotics (streptomycin and penicillin, 50 i.u./ml of each), in an atmosphere of 6% CO_2 in air.

The criterion of action of the drugs tested was the survival rate of the cells, measured as the number of colonies growing on the 7th day after seeding by Pack's method.

The following drugs were used: serotonin antagonists – preparation No. 407 and cyproheptadine, the histamine antagonist bicarphen*, and also serotonin creatinine sulfate and histamine hydrochloride. Freshly prepared solutions of these substances were added to the cell suspensions. Cells were seeded on Petri or Carrel dishes (400 cells per dish, number of dishes in the experiment at each point 10. Each version of the experiment was repeated from 2 to 5 times). After 3 days the medium was replaced by fresh, and on the 7th day of growth colonies were stained with methylene blue and counted.

Amines were determined in the fibroblast extracts by a spectrofluorometric method with purification on a Dowex-50 (from Serva, West Germany) ion-exchange resin in the sodium form [6]. For this purpose fibroblasts were seeded in a dose of 5×10^4 cells/ml medium in 1-liter culture flasks to produce the necessary biomass. Usually the sample contained 100-200 million cells. Samples were taken on the 2nd, 3rd, 4th, and 5th days. The cells were trypsinized and washed with Hanks' solution, then with physiological saline. The cells were extracted with 2% perchloric acid containing 0.5% EDTA, eluted from the columns with 3 N perchloric acid, and determined by two methods: neutral – based on the natural fluorescence of the amines [9], and on fluorescence of the condensation product with orthophthaleic aldehyde [11]. Measurements were made on the MP-4 fluorescence spectrophotometer (from Hitachi, Japan).

EXPERIMENTAL RESULTS

It will be clear from Tables 1 and 2 that histamine and serotonin have no significant effect on cell survival over a wide range of concentrations. Only in the highest of the concentrations used did these substances reduce colony formation a very little. Serotonin proved to be less toxic than histamine: Its blocking action was exhibited in a concentration about six times higher than that of histamine. All the drugs used reduced the survival rate of Chinese hamster fibroblasts and inhibited colony formation. The higher the concentration of the drug in the medium, the more strongly its action was manifested. Bicarphen was most effective from this

* Quinuclidyl-3-di(orthotolyl)carbinol.

standpoint: Its blocking concentrations were 2.5-12 times lower than those of cyproheptadine and preparation No. 407 with identical action.

To study the specificity of action of the drugs experiments were carried out to test the protective action of histamine and serotonin against the corresponding antagonists. The results of these experiments are given in Tables 1 and 2. Histamine, which reduced the survival rate of the cells a little compared with the control, had a distinct protective action against the blocking effect of bicarphen which, in a concentration of 5 μg/ml, inhibited colony formation by 70-75%. Serotonin (Table 2) did not prevent the blocking action of preparation No. 407 in any of the concentrations used. Moreover, after the combined administration of these drugs the survival rate of the cells was depressed even more, i.e., potentiation of the toxic effect of each component was observed.

The results of biochemical analysis showed that extracts of fibroblasts, when treated with orthophthaleic aldehyde, gave maxima of excitation and fluorescence which coincided with those for tryptamine, whereas in the neutral method the maximum of excitation coincided with the maximum of excitation of tryptamine but the maximum of fluorescence coincided with that for serotonin. Fibroblasts thus evidently contain a mixture of indole derivatives, the most important of which are tryptamine and serotonin. Calculations showed that tryptamine was present in an amount of 200-500 ng/10^6 cells (depending on the day of growth), and serotonin in an amount 1 order of magnitude smaller.

Chinese hamster fibroblasts are thus sensitive to several drugs with the action of serotonin and histamine antagonists. Meanwhile these cells contain tryptamine and serotonin and also, according to preliminary evidence, histamine. It can be tentatively suggested that these endogenous physiologically active substances participate in the regulation of growth of fibroblasts.

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