strong DTH reactions in the control and of the absence of any significant changes in the cellular and tissue dynamics during wound treatment with PHA. Positive changes in the cellular and tissue dynamics took place in animals with a weak DTH after application of PHA emulsion ointment. Judging from data in the literature, some workers have attempted to extend the practice of wound treatment with PHA under clinical conditions [7]. In this connection it must be emphasized that the intradermal test for PHA must be carried out for diagnostic (the state of cellular immunity) and also, possibly, for prognostic (the course of wound healing in the post-operative periods) purposes. Treatment of wounds with the PHA preparation is indicated if the intradermal test is negative or weakly positive.

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A MOUSE CELL LINE WITH INHERITED STABLE COLCHICINE RESISTANCE

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KEY WORDS: colchicine; resistance; membrane permeability; heredity.

Resistance of mammalian somatic cells to antitubulins and, in particular, to colchicine and colcemid arises as a rule as a result of changes in permeability of the cell plasma membrane, leading to a decrease in accumulation of the mitostatics [3, 6, 9]. An interesting feature of these membrane changes is their unstable inheritance in a series of cell generations when the cells are cultured under nonselective conditions [1, 2, 9]. It has accordingly been suggested that such changes in membrane permeability may arise not as a result of gene mutations, but because of other genetic changes [1, 2, 9].

The present investigation was devoted to the description of a new subline of mouse cells resistant to colchicine.

EXPERIMENTAL METHOD

Mouse cells of lines L [5] and B-82, a subline of L cells deficient in thymidine kinase [8], were used. The cells were cultured in medium containing 45% Eagle's medium, 45% lactalbumin hydrolysate, 10% bovine serum, and 100 i.u./ml monomycin.

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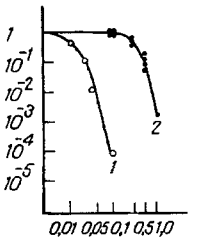

Fig. 1

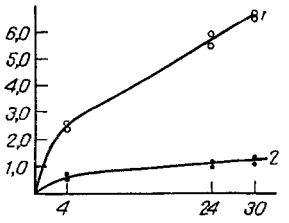

Fig. 2

Fig. 1. Survival rate of B-82 (1) and B-82$^{CH^R}$-9 (2) cells during treatment with different concentrations of colchicine. Abscissa, colchicine concentration (in μg/ml); ordinate, fraction of cells capable of forming colonies in selective medium. Each point corresponds to mean data obtained in one experiment when number of colonies was counted on three dishes.

Fig. 2. Accumulation of ^{3}H-colchicine by B-82 (1) and B-82$_{CH}$CH^R-9 (2) cells incubated for different times with the preparation. Abscissa, time of incubation of cells with ^{3}H-colchicine (in h); ordinate, radioactivity in cells (in thousands of cpm). Each point corresponds to one flask.

The new line, called B-82$^{CH^R}$-9, was obtained by multistage selection during culture of B-82 cells in medium with increasing concentrations of colchicine (from Merck, West Germany) and Tween-80 (from Lawson, England). When 3.5 months had elapsed after the beginning of obtaining the resistant subline, cells capable of propagation in medium with 0.03 μg/ml colchicine and 0.01% Tween-80 were treated for 18 h with 0.3 mg/ml of ethylmethanesulfonate (from Sigma, USA) and, after 7 days, they were transferred to medium containing 0.1 μg/ml colchicine and 0.02% Tween-80. About 2 weeks later, colonies appeared with a frequency of about 1×10^{-5}, and one of them gave rise to the B-82$^{CH^R}$-9 line. The cells of this clone were continuously cultured in medium containing 0.5 μg/ml colchicine.

The survival rate of the cells when treated with different concentrations of colchicine was judged from the efficiency of clone formation by the cells in mitostatic medium, just as previously [1, 2]. Accumulation of ^{3}H-colchicine, ^{3}H-puromycin, ^{3}H-cytochalasin B, ^{3}H-leucine (all from the Radiochemical Centre, Amersham, England), and ^{3}H-colcemid (from New England Nuclear, USA) by whole cells and binding of ^{3}H-colchicine by cell extracts were determined by methods described previously [3, 10].

EXPERIMENTAL RESULTS

The results of experiments to determine the survival rate of cells of lines B-82 and B-82$^{CH^R}$-9 under the influence of increasing concentrations of colchicine are given in Fig. 1. They show that the B-82$^{CH^R}$-9 cells were much more resistant to the mitostatic medium than the wild-type cells. Colchicine, in a concentration of 0.01 μg/ml, reduced the number of cells capable of giving colonies in selective medium by about half. The sensitivity of the B-82 cells to colchicine was thus indistinguishable from the sensitivity of L-cells to this mitostatic substance, which the writers studied previously, for which LD_{50} was 0.011 μg/ml [1]. The survival rate of L and B-82 cells when treated with 0.1 μg/ml colchicine was 3×10^{-6}–9×10^{-5}, whereas in a population of B-82$^{CH^R}$-9 cells in the same concentration 73-87% of cells survived. LD_{50} for B-82$^{CH^R}$-9 cells was about 0.25 μg/ml, or about 25 times higher than LD_{50} for B-82 and L cells.

The study of the mechanisms of resistance of B-82$^{CH^R}$-9 cells to colchicine in five experiments showed that resistant cells accumulate only 10-20% the amount of ^{3}H-colchicine accumulated by the original sensitive B-82 and L cells. The results of one such experiment are shown in Fig. 2. Both the initial rate of entry of the preparation and the maximal amount of it accumulated at the given extracellular concentration were reduced in the resistant subline. In this experiment the B-82$^{CH^R}$-9 cells, during different times of incubation with the preparation (4, 24, and 30 h), accumulated 6.5-6.9 times less of the labeled antitubulin than B-82 cells.

Since the observed decrease in accumulation of ^{3}H-colchicine by resistant cells could be due both to changes in permeability of the plasma membrane and to changes in binding of the preparation with the intra-

TABLE 1. Binding of ^{3}H-Colchicine (in cpm × 10^{-3}) by Homogenates of B-82 and B-82$^{CH^R}$-9 Cells

Dilution of homogenates	Time of incubation with 2.5 μm ^{3}H-colchicine, min	B-82	B-82$^{CH^R}$-9
Original ($2.5 \cdot 10^5$ cells in sample)	0	1,8±0,1	2,8±0,6
	30	23,0±0,3	18,7±0,5
	60	26,5±2,2	20,2
1:2	0	1,8±0,2	3,2±0,5
	30	11,1±0,1	9,6±0,1
	60	11,6±0,5	10,3±0,1-2,4
1:4	0	2,0	
	30	5,4±0,2	5,1±0,5
	60	5,7±0	4,6±0,5

cellular target (tubulin), binding of the labeled antitubulin with extracts of B-82$^{CH^R}$-9 and B-82 cells was compared. The results in Table 1 show that homogenates from the same quantity of sensitive and resistant cells bind about equal amount of ^{3}H-colchicine. On dilution of the homogenates, the quantity of label bound decreased as a linear function. This indicates that during incubation (30 min-1 h) practically all the tubulin succeeded in binding with the preparation. The ratio of label bound by the homogenates of the B-82$^{CH^R}$-9 cells was 1.06-1.35. This very small difference, which was not statistically significant, cannot of course explain the very considerable difference (5-10 times) in accumulation of the labeled antitubulin.

Yet another argument in support of the view that resistance of B-82$^{CH^R}$-9 cells to colchicine is due to the lower permeability of the plasma membrane is given by the results of experiments to compare accumulation of a series of labeled preparations by cells sensitive and resistant to colchicine. On incubation with the preparations for 4-24 h it was found that B-82 and L cells accumulate 2.1 ± 0.05 times more ^{3}H-puromycin 2.5 ± 0 times more ^{3}H-cytochalasin B, and 2.2 ± 0.5 times more ^{3}H-colcemid than B-82$^{CH^R}$-9 cells. Meanwhile the resistant cells accumulated almost the same quantity of ^{3}H-leucine or rather more (by 10%). The decrease in accumulation of substances not bound with the tubulin (puromycin, cytochalasin B) by the resistant cells is a characteristic feature of colchicine-resistance due to reduced permeability of the plasma membrane [4, 6].

Inheritability of the observed changes is an important criterion in the study of the genetic nature of this feature. Stability of inheritance of colchicine resistance was determined in the new B-82$^{CH^R}$-9 line. It was found that after culture without a selective agent for 3 and 6 months the sensitivity of the cell population to the mitostatic was unchanged. Curves of survival rate of the cells under the influence of colchicine, determined after culture without the mitostatic medium for the specified period of time, did not differ from the curve of survival of cells continuously cultured with colchicine, shown in Fig. 1.

When selecting cells with the aid of colchicine and the substance Tween-80, which increases the permeability of the plasma membrane for the mitostatic substance, it was expected that mutants for tubulin would be selected, but in fact, "ordinary" variants with modified permeability for colchicine were obtained. Canadian workers, using exactly the same method of selection, have recently isolated a number of clones of Chinese hamster cells of the CHO line with modified tubulin [7]. These mutants for tubulin differ from the line described above in a number of properties. Whereas accumulation of labeled mitostatic substances in tubulin mutants by whole cells was not modified, binding of the labeled antitubulin by cell extracts was reduced by 50-80%, and cross resistance was observed only to substances binding with tubulin; line B-82$^{CH^R}$-9 was characterized by a decrease in accumulation of ^{3}H-colchicine by whole cells of 80-90%, by unchanged binding of the preparation by cell homogenates, and by cross resistance to substances not binding with tubulin. Selection in a selective medium containing colchicine and Tween-80 can evidently lead to the isolation both of cells with modified protein of their microtubules and of variants with modified permeability of their plasma membrane.

However, the new B-82$^{CH^R}$-9 line differs significantly from other mouse lines and clones with lowered membrane permeability for colchicine obtained previously by the fact that its phenotype is stable. Even after culture of cells of this line in medium without the mitostatic for 6 months, resistance to colchicine still remained at its original level, whereas in cells studied previously resistance to the mitostatic was lost equally in clones with a high and low level of resistance, at the rate of about 2×10^{-2} per cell per generation [1, 2]. Different genetic events may probably lead to the development of reduced membrane permeability.

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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