distribution of biogenic amines and associated metabolites in dog brain, have concluded that brain areas with lower 5 HT concentrations had a higher efficiency of utilization of this amine as evidenced by a lower 5 HT/5 HIAA ratio. In other words, from these authors[6], it seems that a low 5 HT/5 HIAA ratio means a high efficiency of 5 HT utilization. In the present work the fact that, when compared to reference values (HP = 1 ATA; TW = 14 °C), the ratio is increased with Tw but not with HP can mean that in fish, a high efficiency of 5 HT utilization is more dependent on temperature than pressure. From the results it may be supposed that the changes in behavior and the motor hyperactivity (which correlates well with 5 HT levels in fish brain[4]) observed under pressure are not due to changes in the content of indolamine neurotransmitter in brain but perhaps to an HP effect on receptor sites[8].

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0014-4754/85/111429-02$1.50 + 0.20/0

Selective protection of cells against X-irradiation. Isoproterenol protects only those cells that possess β-adrenoreceptors

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Summary. In mixed culture of Chinese hamster fibroblasts, clone 431, and transformed murine L fibroblasts, clone B-82, isoproterenol was found to protect only 431 cells against ionizing radiation. It was shown that 431 cells, in contrast to B-82 cells, possess β-adrenoreceptors, and the radioprotective effect of isoproterenol can be realized only if this agent interacts with β-adrenoreceptors coupled with the cAMP system. Since malignization often causes the disappearance of β-adrenergic and other hormone receptors, the combined culturing and irradiation of the cells studied can be regarded as a model of the growth of malignant cells (B-82) among normal tissue cells (431 cells) under conditions of radiation therapy. A possibility of selective protection against radiation damage of normal tissue cells, with retention of the former radiosensitivity of tumor cells, is discussed.
Key words. Radioprotection; cell cultures; isoproterenol; β-adrenoreceptor; cellular cAMP concentration.

At present methods for the enhancement of the efficacy of radiation therapy are chiefly based on the knowledge and application of the fact that oxygenation in the tumor is reduced as the result of an imbalance between cell growth and vascularization. The development of hypoxic conditions in the tumor offers a possibility of using radiosensitizers of hypoxic cells, such as metronidazol, misonidazol, etc. However, the efficacy of applying these radiosensitizers is diminished by the fact that only 10–20% of the tumor cells are hypoxic, and also because the fractionated irradiation commonly used in clinical practice sharply reduces the effect of radiosensitizers[9]. Thus, it is necessary to investigate other peculiarities differentiating tumor cells from normal cells[12]. It is well known that cell malignization often leads to the disappearance or 'masking' of receptors of some hormones and to the loss of the response of the cell adenylate cyclase system to these hormones[6,7,18,25]. We have demonstrated earlier that adenylate cyclase stimulation through the hormone receptors leads to a decrease in the cell radiosensitivity[4,5,22], and that the radioprotective effect is connected with activation of the cAMP system[23]. It follows from the results of these works that the chain of events listed below:

1) binding of the adenylate cyclase activator to the receptor;
2) adenylate cyclase stimulation;
3) cAMP accumulation;
4) intensification of cAMP-dependent phosphorylation,

leads to the increase in cell radioresistance. The absence of one required unit, that of the receptor binding to the agonist, serves as an obstacle for reducing the cell radiosensitivity under the effect of this agonist. It is suggested that introduction of the agonist into the mixture of two types of cells:

1) possessing a receptor to it and capable of increasing the cAMP concentration in response to this agonist and
2) devoid of receptor

would lead to selective protection of cells of the first type. This suggestion was experimentally confirmed in this work for mammalian cells (cultured in vitro) possessing and devoid of β-adrenergic receptors.

Materials and methods. Cells. Chinese hamster fibroblasts B 11 dii FAF-28, clone 431, were obtained from the Institute of Developmental Biology, the USSR Academy of Sciences, and murine fibroblasts L, clone B-82, from the Institute of Molecular Biology, the USSR Academy of Sciences. The cells were grown as monolayers at 37 °C in a medium containing 45% Eagle's medium, 45% medium No. 199, 10% bovine serum, penicillin (100 U/ml) and streptomycin (100 μg/ml). When they reached the late log growth phase the cells were washed off with 0.02% EDTA solution and suspended in Hanks' solution.

Determination of cell radiosensitivity. The cell suspension (500 cells in 1 ml in case of separate irradiation or 400 cells of 431 clone plus 700 cells of clone B-82 in 1 ml in case of combined irradiation) were subjected to X-irradiation in glass vials in 0.5–6.0 Gy doses (0.5 and 1.0 Gy/min, 200 kV, 15 mA, filters: 0.5 mm Cu + 1.0 mm Al). Following the irradiation, we added a growth medium containing 30% bovine serum. Cell survival was determined by the colony-forming ability[17]. Macrocolonies were counted 10 days after irradiation. 431 and B-82 cells form quite different macrocolonies. Macrocolonies of B-82 cells are flat and of a regular round shape; the cells are triangular or sometimes rhomboid. Macrocolonies of 431 cells are convex, multilayered of irregular shape; the cells are elongated, spindle-shaped. Due to these differences between the cell colonies it is possible to determine the radiosensitivity of these cells after their combined irradiation and culturing. The radioprotective capacity of the agent under study was judged by the changes in D_0 dose of the

irradiated cells, and the dose modifying factor (DMF) was calculated from the ratio of D_0 doses in the presence and absence of the agent.

Determination of intracellular cAMP content. After incubation of the cells with d,l-isoproterenol (Sigma), d,l-propranolol (Sigma) or prostaglandin E_1 (Institute of Chemistry, Estonian Academy of Sciences) at 37 °C they were precipitated by centrifugation (800 × g, for 3 min), resuspended in 0.5 ml of 4 mM EDTA (Serva) and deproteinized at 100 °C for 5 min. cAMP concentration was determined in the cooled extract using a cAMP assay kit (Amersham) by the modified method described by us previously[22].

Determination of adenylate cyclase activity in B-82 cells. The cells were lysed in a 20-fold volume of 5 mM Tris-HCl (pH 8.1), containing 1 mM $MgCl_2$, 0.5 mM EDTA, 2 mM 2-mercaptoethanol (Serva) for 20 min at 4 °C, and then homogenized in a Potter-Elvehjem homogenizer (30 strokes of pestle). The nonlysed cells and nuclei were separated by centrifugation (800 × g, for 1 min); the rough membrane fraction was precipitated by recentrifugation (20,000 × g, for 20 min). The pellet was resuspended in the buffer mentioned above. Adenylate cyclase activity was determined by the method described earlier[23] with the use of [^{14}C]-ATP (UVVVR) as a substrate with the subsequent purification (chromatography, electrophoresis) of the [^{14}C]-cAMP formed.

Determination of β-adrenoreceptors in 431 cells. The cells were lysed in a 20-fold volume of bidistilled water for 10 min at 4 °C. Lysis was stopped by adding an equal volume of 10 mM Tris-HCl (pH 7.6) containing 5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM 2-mercaptoethanol and the preparation obtained was centrifuged (25,000 × g, for 20 min). The pellet, representing a rough membrane fraction, was resuspended in the above mentioned buffer, and used for equilibrium binding with 0.6–12.0 × 10^{-9} M 1-[propyl-4,6-^{3}H]-dihydroalprenolol ([^{3}H]-DHA) (Amersham) for 30 min at 25 °C according to the method described previously[26]. Specific binding was calculated as the difference between the total and nonspecific (in the presence of 10 μM d,l-propranolol) binding. After the linearization of data in Scatchard plots the maximum number of specific binding sites for [^{3}H]-DHA was calculated[24].

Other methods. Radioactivity was determined using a liquid scintillation counter Marck-II (Nuclear Chicago). Protein was determined according to Lowry et al.[15]. Regression equations for the linear parts of the survival curves were computed by the least squares method[12]. All data represent means ±SE.

Results. We discovered that Chinese hamster fibroblasts (clone 431) are able to bind specifically [^{3}H]-dihydroalprenolol ([^{3}H]-DHA) which binds to β-adrenoreceptors. Linearization of the [^{3}H]-DHA adsorption isotherm using a Scatchard plot offered the possibility of determining the maximum number of [^{3}H]-DHA specific binding sites (350 fmoles/mg protein), corresponding to the number of β-adrenoreceptors[24], and the dissociation constant of the [^{3}H]-DHA-β-receptor complex (13 nM). Incubation of 431 cells with 1 μM isoproterenol, a specific β-agonist, for 5 min at 37 °C induced a 1.8-fold increase in intracellular cAMP content; β-antagonist propranolol (1 μM) prevented the effect caused by isoproterenol (see table). When isoproterenol (1 μM) was added to 431 cells 5 min before X-irradiation, a 1.5-fold increase in cell radioresistance was recorded; propranolol (1 μM) blocked the radioprotective effect of isoproterenol (fig. 1). Propranolol alone had no effect on cAMP level and radiosensitivity of the cells (data not shown). Changes in concen-

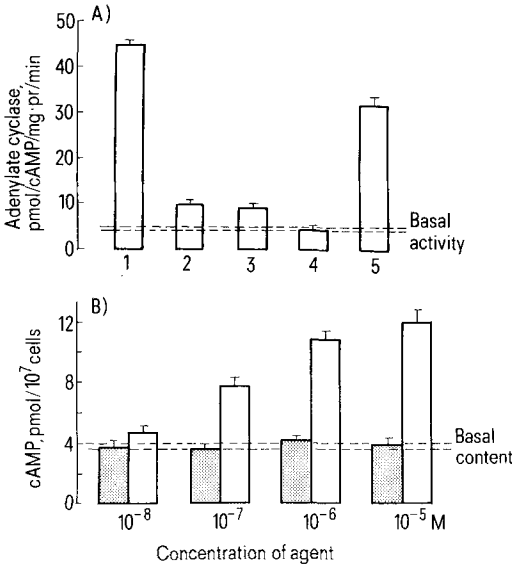

Figure 2. Adenylate cyclase activity (A) and cAMP content (B) in B-82 cells after experimental treatment. A): *1* 10 mM NaF, *2* 0.1 mM GTP, *3* 0.1 mM GTP + 10 μM isoproterenol, *4* 10 μM isoproterenol, *5* 10 μM prostaglandin E_1. B): solid bars: isoproterenol, open bars: prostaglandin E_1. Results of four experiments are presented.

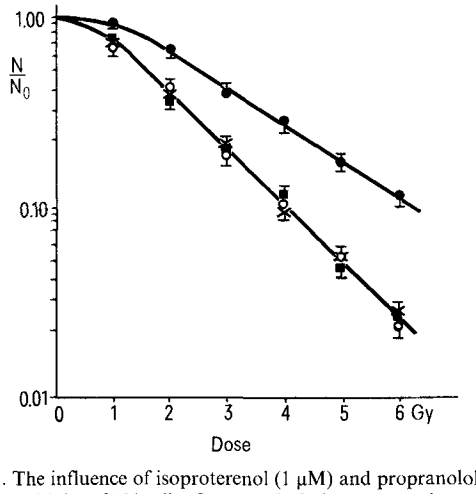

Figure 1. The influence of isoproterenol (1 μM) and propranolol (1 μM) on radiosensitivity of 431 cells. ○, control; ●, isoproterenol; ×, propranolol + isoproterenol; ■, isoproterenol + washing. Incubation with isoproterenol: 5 min; preincubation with propranolol: 3 min. Results of the three similar experiments are presented.

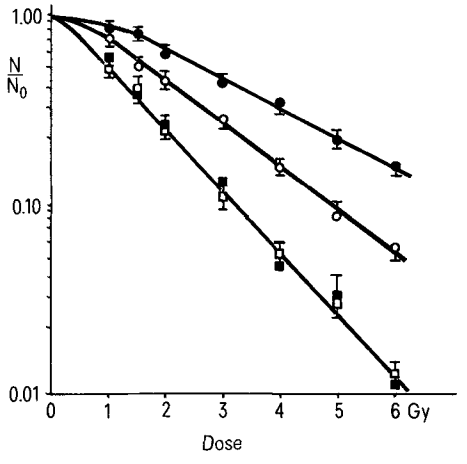

Figure 3. Survival of 431 and B-82 cells after their combined X-irradiation in the presence and absence of 1 μM isoproterenol. ○, 431 cells, control; ●, 431 cells, isoproterenol; □, B-82 cells, control; ■, B-82 cells, isoproterenol. Results of one of the four similar experiments are presented.

tration and cell radiosensitivity were eliminated when isoproterenol was washed off (table; fig. 1).

As distinct from 431 cells, transformed murine L-fibroblasts (clone B-82) possess no β-adrenoreceptors[16]. Adenylate cyclase of B-82 cells responded to 0.1 mM GTP and 10 mM NaF, but was not activated with isoproterenol (fig. 2a); isoproterenol showed no effect on the cAMP content in intact B-82 cells (fig. 2b). In a mixture of 431 and B-82 cells isoproterenol protected 431 cells (DMF = 1.43, fig. 3) against X-irradiation but failed to modify radiosensitivity of B-82 cells (fig. 3). B-82 cells are known to possess receptors for prostaglandin E_1 (PGE_1)[3], and therefore PGE_1 causes both an increase in adenylate cyclase activity of the membrane preparations (fig. 2a) and a rise in the cAMP level in intact cells (fig. 2b). Earlier we have shown[4] that B-82 cells can be protected by PGE_1; DMF = 1.34 after 5 min incubation of cells with $5 \cdot 10^{-7}$ M PGE_1.

Thus, the absence of β-adrenoreceptors in B-82 cells predetermined the absence of an effect of a β-agonist (isoproterenol) on the radiosensitivity of these cells.

Discussion. As shown by us earlier, a specific β-agonist isoproterenol protected isolated mammalian cells against ionizing radiation and caused an increase in intracellular cAMP content; the concentration dependencies of the cAMP-stimulating and radioprotective action of isoproterenol were parallel[10,22]. Results reported in the present work demonstrate the competition between β-agonist isoproterenol and β-antagonist propranolol both in protection of cells against X-irradiation (fig. 1) and in the effect on the cAMP system of 431 cells (table). In cells devoid of β-adrenoreceptors (B-82 cells) isoproterenol is incapable of expressing its radioprotective potential (fig. 3). And what is more, recently we have demonstrated that the radioprotective effect of isoproterenol on 431 cells can be prevented by functional 'uncoupling' of β-adrenoreceptors from adenylate cyclase[5]. Such 'uncoupling' is a result of the desensitization of the cAMP system to isoproterenol induced by prolonged incubation of cells with β-agonist[24].

Thus, the present and previous data give evidence for the participation of β-adrenoreceptors in the radioprotection of cells by isoproterenol. The loss of a β-receptor (B-82 cells) prevents the isoproterenol action both on the cell adenylate cyclase system and on its radiosensitivity.

Adenylate cyclase of many tumor cells is known to lose the capacity of being stimulated with a β-agonist. For example, murine kidney carcinoma cells (RAG)[9], human carcinoma of the uterine cervix (HeLa)[13,25], murine adrenocarcinoma (Y-1)[20], human leukemic lymphocytes[18], Friend erythroleukemia[13]. The absence of β-adrenergic stimulation of adenylate cyclase was shown to be due to the loss of β-receptors by these cells. It should be noted that induction of transformed cells to differentiation led to the appearance of β-adrenoreceptors in the membrane and to the restoration of β-agonist stimulation of adenylate cyclase[13,19,25].

The cAMP content of 431 cells

Experiment	pmoles cAMP/10^6 cells	Number of experiments
1 Control	3.28 ± 0.20	9
2 1 μM isoproterenol	6.03 ± 0.41 ($p < 0.01$)	10
3 1 μM isoproterenol + 1 μM propranolol	3.50 ± 0.21	5
4 1 μM propranolol + 1 μM isoproterenol	3.56 ± 0.27	5
5 1 μM isoproterenol + washing	3.41 ± 0.25	6

Cells were incubated with isoproterenol for 5 min. In experiment 3 propranolol was given simultaneously with isoproterenol; in experiment 4 cells were preincubated with propranolol for 5 min and then isoproterenol was added. In experiment 5 cells, after incubation with isoproterenol, were precipitated by centrifugation (800 × g, 3 min), resuspended in Hanks' solution, and incubated for 5 min.

The combined culturing of B-82 and 431 cells was considered as a model for the growth of malignant cells (in this case of cells B-82) among normal tissue cells (431 cells). Isoproterenol treatment before irradiation of a combined cell culture reduced the radiosensitivity of 431 cells alone, without altering the radiosensitivity of B-82 cells (fig. 3).

Malignization causes the disappearance or reduction of the number not only of β-adrenoreceptors, but also of the other membrane receptors coupled with adenylate cyclase[6,7]. It is also known that such adenylate cyclase activators as PGE_1, serotonin, histamine, etc. can protect mammalian cells in vitro against ionizing radiation[1,2,4,14,21]. It can be suggested that the loss of receptors to any of these agents would lead to the loss of its radioprotective potency. Thus, the changes in the number of receptors in the malignant cells can be used for selective protection of healthy tissue in the radiation therapy of tumors. Radiosensitivity of tumor cells may be expected to remain at the former level, whereas the surrounding normal cells will become more radioresistant.

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