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ACTIVE COBALAMIN TRANSPORT IN L-1210 MOUSE LEUKEMIA CELLS

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A decisive role in the mechanism of the supply of cobalamin coenzyme precursors to cells, which are essential for their normal proliferation and maturation, is played by the formation of a transcobalamin-II cobalamin complex (TC-II+Cbl) with the membrane surface receptor. Specific receptors of this type have been found on cells of the villi of the mature human placenta, the spleen, normal hepatocytes, and L-1210 mouse leukemia cells [6, 12]. Changes in functional activity of TC-II receptors of the cell membrane surface may be responsible for differences in the intensity of cobalamin transport [1].

The effect of temperature and ionic composition of the medium on affinity of hormone receptors for the ligand is known [10]. The number of membrane surface receptors may vary depending on the concentration of the ligand in the medium and in the course of cell differentiation [7, 11]. However, the factors changing the number of TC-II receptors on the surface membrane of tumor cells are not yet known. We therefore investigated the effect of temperature, cobalamin concentration in the medium, and their intracellular concentration on the level of binding of radioactive [TC-II+Cbl] with surface receptors of L-1210 mouse leukemia cells. The functional activity of the receptors and endocytosis of cobalamins were investigated during the mitotic cell cycle in vitro.

EXPERIMENTAL METHOD

Experiments were carried out on L-1210 mouse leukemia cells isolated from ascites fluid on the 7th day after intraperitoneal injection of 2×10^6 mouse spleen cells of line DBA/2. Because of the absence of species-specific differences in protein transport, in order to saturate the surface receptors we used saturated ^{57}Co-cyanocobalamin (^{57}CoCNCbl) TC-II, obtained by special treatment of rabbit serum [4], as the standard. For quantitative determination of the receptors we used the ability of the proteolytic enzyme papain to remove protein molecules from the surface membrane cells, by cleaving their polypeptide chain [2]. The successive stages of determination were as follows: saturation of the cell surface receptors with the radioactive complex, separation of ligand-receptor complexes with radioactive label by papain from the cell surface membrane, determination of radioactivity on a γ-counter on solubilized receptors and inside the cells. Receptors of leukemic cells $(1.0\text{-}1.5) \times 10^7$ were estimated during short-term culture (between 5 and 100 min) in Eagle's medium with 10% standard serum, containing [TC-II+^{57}CoCNCbl]. The leukemic cells were analyzed in the experiments directly after isolation from ascites fluid and after their preliminary incubation (5 h, 37°C) in medium deficient in cobalamins, but containing TC-II. The pool of endogenous cobalamins in the L-1210 cells was determined by a microbiological method with *E. coli* 113/3 [1]. Receptors of the cell membrane surface were estimated at different periods of the mitotic cycle during longer-term culture (25 h) in the same medium with the addition of 10% embryonic calf serum. Binding of radioactive TC-II with receptors, accumulation of ^{57}CoCNCbl in the cytoplasm, and the number of cells synthesizing DNA were investigated at the same times. In this series of experiments, with the aim

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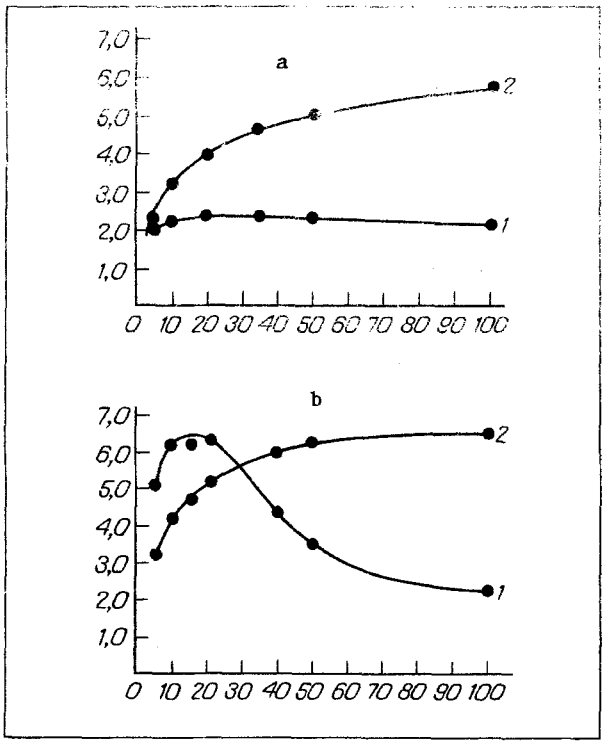

Fig. 1. Binding of [TC-II+^{57}CoCNCbl] complex with surface membrane receptors (1) and accumulation of ^{57}CoCNCbl in cytoplasm (2) of L-1210 leukemia cells differing in their content of endogenous cobalamins. a) 249 pg/10^6 Cells, b) 125 pg/10^6 cells. Abscissa, time of incubation of cells (in min); ordinate, content of radioactive label (in pg/10^6 cells). Each point on graph indicates mean value of measurement of three parallel samples.

of achieving synchronization, the cells were kept beforehand for 5 h at 4°C, then introduced into Carrel flasks (2×10^7 cells to 5 ml of medium), and incubated at 37°C. Every hour during incubation the cells were analyzed by the double label method: ^{57}CoCNCbl to estimate the number of receptors and ^{3}H-thymidine to determine the S-phase. Incorporation of ^{3}H-thymidine into DNA of leukemic cells was determined by the method in [9]. The ^{3}H-thymidine used was of Soviet manufacture (specific radioactivity 780 TBq/mmole, 0.064 g/liter) and was added to the samples 1 h before determination, and counted on a Mark-3S β-counter with ZhS-8 scintillator. The rest of the cells of the same sample were used to determine TC-II receptors on the surface membrane and the ^{57}CoCBCbl concentration in the cytoplasm. The ^{57}CoCNCbl was obtained from "Amersham" and its radioactivity was 389 kBq, 0.05 μg/ml. The samples were counted on MAG-510 γ-counter ("Bertold").

EXPERIMENTAL RESULTS

The first phase of specific binding of the [TC-II+^{57}CoCNCbl] complex with the surface membrane receptor of the leukemic cells ended with its internalization and accumulation of ^{57}CoCNCbl in the cytoplasm. To study active cobalamin transport into the cells we undertook a differential count of radioactivity on receptors and in the cytoplasm of L-1210 tumor cells. According to the results, the level of binding of the radioactive complex with membrane receptors was unchanged when the cells were cooled (4°C, 90 min). However, endocytosis, as an energy-dependent process, was inhibited by 90-95%, and accordingly, as a result of uncoupling of the mechanisms of binding of the complex and its internalization, accumulation of ^{57}CoCNCbl in the cells did not take place. During short-term culture of the tumor cells with the radioactive complex of blood serum, binding with membrane receptors reached a maximum after 15 min. This time, incidentally, was similar to maximal binding of the ligand by receptors of phorbol esters [8]. The level of binding of the [TC-II+^{57}CoCNBl] complex by specific surface membrane receptors differed in cells differing in their content of endogenous cobalamins. In cells with a higher content (249 pg/10^6), removed directly from ascites fluid, the level of binding of the ligand remained virtually constant during a long period of observa-

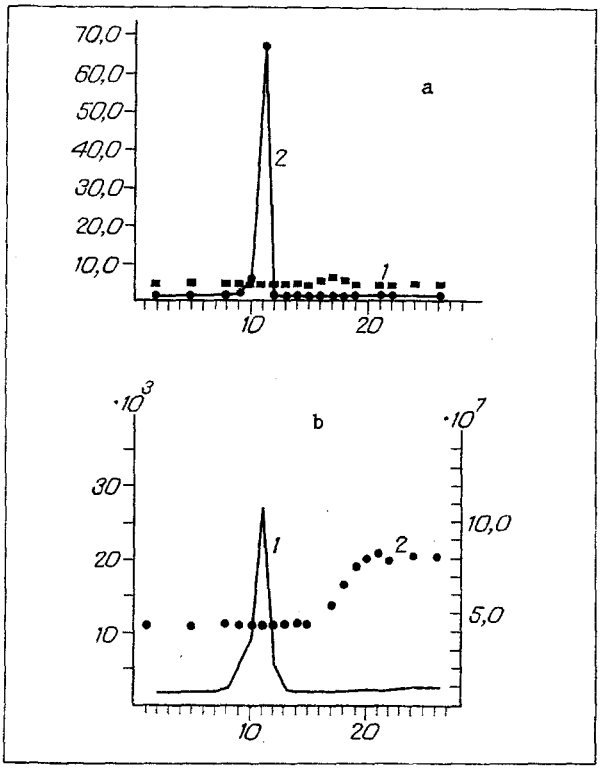

Fig. 2. Accumulation of ^{57}CoCNCbl on TC-II receptors and in cytoplasm of L-1210 leukemia cells at different periods of mitotic cycle. a: Abscissa, duration of culture (in h); ordinate, accumulation of ^{57}CoCNCbl (in pg/10^6 cells): on receptors (1), in cytoplasm (2); b: abscissa, duration of culture (in h), ordinate: on left – incorporation of ^{3}H-thymidine (1, cpm); on right, number of cells in samples (2). Graphs plotted from results of three series of experiments.

tion. Under these circumstances ^{57}CoCNCbl was found in the cell cytoplasm in increasing amounts (Fig. 1). Preincubation of the cells in medium without cobalamin caused reduction of the intracellular pool of endogenous cobalamins (125 pg/10^6 cells), although in the control, on incubation in ascites fluid, no such decrease was observed. The level of binding of the radioactive complex by surface receptors was much higher in cells with a lower content of cobalamins. During their culture, with saturation of the cytoplasm with ^{57}CoCNCbl, binding of the ligand was reduced. These results indicate an important role for the ligand in the regulation of the number TC-II-receptors of the cell surface membrane and they correlate with existing data on other classes of receptors [5].

The duration of the mitotic cycle of L-1210 cells depends on several factors and, according to data obtained by different workers, it varies within wide limits [3]. In our investigations we observed doubling of the number of synchronized cells during culture for 20 h with a constant concentration of cobalamins and TC-II, which ruled out any effect of the medium on the number of receptors discovered (Fig. 2). The level of binding of the radioactive complex with surface membrane receptors of L-1210 cells was unchanged during long-term culture. Meanwhile, accumulation of ^{57}CoCNCbl in the cytoplasm took place only within a certain period. Internalization of the radioactive complex was observed between 7 and 15 h of cell culture, at a time of intensive incorporation of ^{3}H-thymidine into the DNA of these cells (Fig. 2).

The following conclusion can be drawn from the results relating to cobalamin transport into leukemic cells, cultured under different conditions. The number of TC-II receptors on the surface membrane of leukemia cells determines the intracellular cobalamin concentration. The number of receptors is independent of changes in temperature of the culture medium or the phase of the cell cycle. Under these circumstances endocytosis of cobalamins is effected only during the S-phase of the mitotic cycle.

The rate of penetration of cobalamins into the cell is evidently controlled with the aid of modulation of the number of TC-II membrane receptors in response to external stimuli. Regulation of this rate at the endocytosis level is unlikely, for internalization of the complex within a definite period of the cell cycle rises extremely rapidly, a characteristic feature of several cooperative processes acting in accordance with the "all or nothing" principle.

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RATE OF GROWTH OF HUMAN MELANOMA CELLS IMPLANTED INTO IMMUNOSUPPRESSED MICE IN A FIBRIN CLOT

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Xenografts of human tumors are the most adequate model with which to study human cancer. To evaluate the activity of therapeutic agents the method of implanting fragments of human tumors [2] or cells from culture, incorporated into a fibrin clot (FC) [7], beneath the renal capsule (subcapsular implantation, SCI) of mice is used. It is considered that comparing the dimensions of the tumor in control and experimental mice provides a basis for assessing the activity of such preparations, but infiltration of the tumor by the host's cells in immunocompetent mice may disturb the reliability of these measurements [3]. To prolong the viability of the implant, immunosuppression of the recipients can be used [4].

It is advisable to use tumors such as human melanoma BRO, which proliferate rapidly after implantation in nude [5] and immunosuppressed mice [1, 6]. This paper describes the use of FC with BRO cells obtained from culture in order to study growth of the human tumor in mice.

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