9. C. Beauchamp and I. Fridovich, Anal. Biochem., 44, 276 (1971).
10. E. W. Kellogg and I. Fridovich, J. Gerontol., 31, 405 (1976).
11. B. E. Leibovitz and B. V. Siegel, J. Gerontol., 35, 45 (1980).
12. S. Marklund, I. Nordensson, and O. Bäck, J. Gerontol., 36, 405 (1981).
13. H. Nohl, D. Hegner, and K.-H. Summer, Mech. Ageing Develop., 11, 145 (1979).
14. J. M. Tolmasoff, T. Ono, and R. G. Cutler, Proc. Nat. Acad. Sci. USA, 77, 2777 (1980).

# CHANGES IN THE PROTEIN-SYNTHESIZING SYSTEM OF HeLa CELLS IN CULTURE IN THE PRESENCE OF TRACE ELEMENTS

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Zinc, nickel, cobalt, cadmium, and fluorine belong to the group of trace elements, because their concentration in the body does not exceed $10^{-3}$-$10^{-12}$%. Of these elements zinc, nickel, cobalt, and fluorine are considered essential for life. For instance, zinc activates about 120 enzymes concerned with different aspects of cell metabolism [15]. There is evidence that nickel can increase and depress activity of certain enzymes both in vivo and in vitro. Cobalt is a component of vitamin $B_{12}$. In addition, within a certain concentration range, this trace element can block sulfhydryl groups of certain structural proteins and enzymes [3]. The action of fluorine on the cell varies, for it behaves not only as an inhibitor of many enzyme systems, but also as a powerful activator, unique among the trace elements, of the metabolically important enzyme adenylate cyclase [2]. Cadmium belongs to a group of trace elements which are toxic for the body even in very low concentrations [4]. It often behaves as an antagonist of zinc, for they compete with each other for certain intracellular ligands. However, it must be remembered that the subdivision of trace elements into essential, neutral, and toxic is very conventional, for all essential elements become toxic when they reach certain concentrations, and the differences between doses in which they are useful and dangerous may be small.

The aim of this investigation was to study the state of the protein-synthesizing system of HeLa cells in culture in the presence of certain trace elements.

## EXPERIMENTAL METHOD

The cytopathic action of zinc, nickel, cobalt, cadmium, and fluorine was studied in the presence of maximal allowable concentrations (MAC) adopted for liquid media, in the form of $ZnSO_4$, $NiCl_2$, $CoCl_2$, $CdCl_2$, and NaF. These concentrations, calculated on the basis of the content of the ion of the trace element in the molecule, were (in $\mu$g/ml): $Zn^{++}$ 1, $Ni^{++}$ 0.1, $Co^{++}$ 1, $Cd^{++}$ 0.01, and $F^-$ 1.5. Sodium sulfate and chloride, in equimolar concentrations, calculated as sulfate and chloride ions, were added to the control cultures. Cultures without addition of salts also were studied (background control). The action of the trace elements on the cell culture was investigated for 2, 4, and 24 h. Charactistics of the method of culture, treatment of the glassware, and conduct of the experiments were described previously [1]. The dynamics of RNA and protein synthesis were studied by autoradiography. For this purpose, 30 min before the end of incubation with the elements to be studied, [$^3$H]uridine (80 mBq/ml, specific radioactivity 1040 GBq/mmole) or [$^3$H]leucine (200 mBq/ml, specific radioactivity 4 GBq/mmole) was added to the cultures of HeLa cells. Autoradiographs were prepared in the usual way and the number of tracks counted above the whole cell ([$^3$H]leucine) or above a unit of area ([$^3$H]uridine). To determine the content of ribosomal RNA (rRNA) and total protein two series of experiments were carried out with staining for RNA by Einarson's method with gallocyanin and chrome alum, and with Naphthyl Yellow for total protein. Staining for rRNA was preceded by treatment of the cells with DNAse (from Reanal, Hungary) for 2 h at 37°C. Concentrations of metabolites were determined by the logarithmic screen method on an MIF integrating photometric microscope at wavelengths of 547 nm (rRNA) and 436 nm (total protein). All numerical data were analyzed on the Nairi computer.

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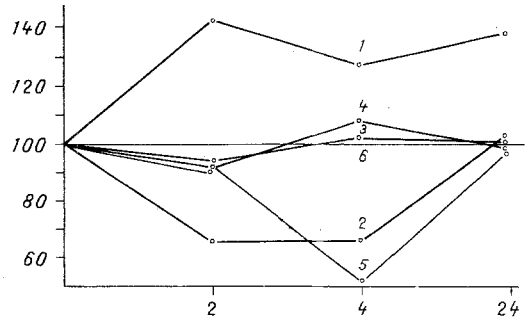


Fig. 1

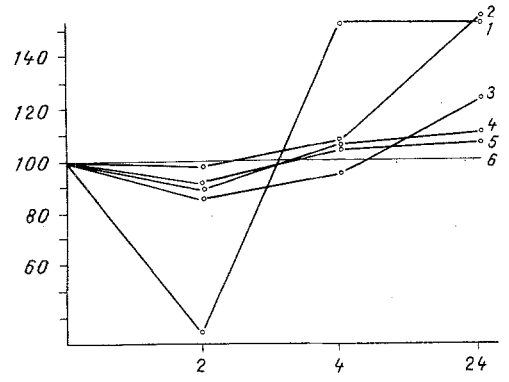


Fig. 2

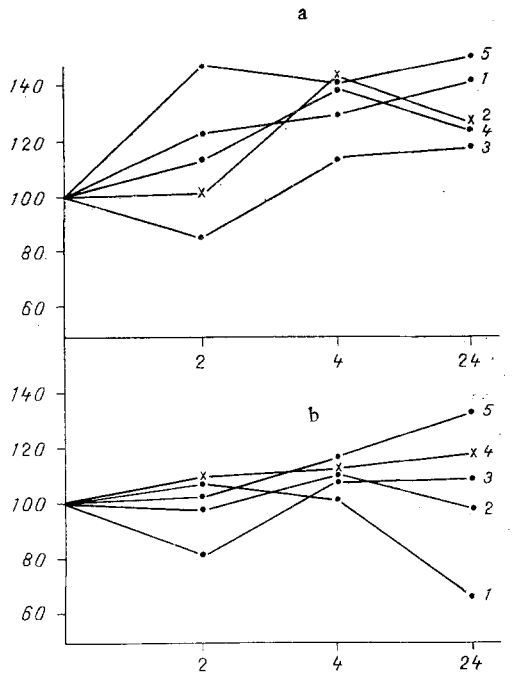


Fig. 3

Fig. 1. Dynamics of incorporation of [$^3$H]uridine into HeLa cells on incubation with MAC of trace elements. Abscissa, duration of experiment (in h); ordinate, number of tracks above a unit area (in percent of control). 1) $Zn^{++}$, 2) $F^-$, 3) $Cd^{++}$, 4) $Co^{++}$, 5) $Ni^{++}$, 6) control.

Fig. 2. Dynamics of incorporation of [$^3$H]leucine into HeLa cells on incubation with MAC of trace elements. Legend as to Fig. 1.

Fig. 3. Change in rRNA and total protein content in cells during treatment with MAC of trace elements. Abscissa, duration of experiment (in h); ordinate: a) rRNA concentration (in percent of control), b) total protein (in percent of control). Remainder of legend as to Fig. 1.

## EXPERIMENTAL RESULTS

The autoradiographic data showed that variation in such integral parameters of cell function as the level of synthesis of total fast-labeled RNA and total protein in fact do take place during incubation of the HeLa cell culture with trace elements. For instance, changes in RNA synthesis in the cell are more marked under the influence of the MAC of zinc: After incubation for 2 h this process was inhibited on average by 65%, whereas after 4 h, on the other hand, an increase in transcription by almost 50% was observed compared with the control, and subsequently this process became stabilized. Nickel, cobalt, and cadmium induced very small fluctuations of transcription during the experiment, differing from the control level on average by 4-20%. There is evidence in the literature that cadmium, in a concentration of 1.124 μg/ml, selectively inhibits synthesis of low-molecular-weight types of RNA against a background of suppression of its synthesis [7], whereas at the same time it stimulates synthesis, by messenger RNA (mRNA) of the proteins which are carriers of this trace element (7.868 μg/ml) [8]. Inhibition of RNA synthesis by certain nickel compounds also have been described [5]. During incubation of cells with the MAC of fluorine, a sharp increase of about 50% in RNA synthesis was observed after 24 h of exposure (Fig. 1).

Modification of protein synthesis in cells was most marked on incubation of the culture with MAC of zinc, nickel, and fluorine. Zinc, for instance, increased uptake of [$^3$H]leucine on average by 30-40% compared with the control. In a survey, Hsu [10] analyzed data showing a general tendency for incorporation of labeled amino acids into various protein fractions to decrease in zinc-deficient animals. Addition of nickel to the incubation

medium, on the other hand, sharply inhibited incorporation of the label, especially after an exposure of 4 h (Fig. 2). Inhibition of protein synthesis in the first stages of the experiment (2 and 4 h) also was observed with the MAC of fluorine. However, just as with nickel, cells incubated with fluorine can restore their protein synthesis almost up to the control level by the end of 24 h. According to Holland [9], suppression of protein synthesis is a feature typical of the action of fluorine on all cell types, but most workers have observed this effect in concentrations much higher than that used in the present study. When cadmium and cobalt were used, only small variations of incorporation of labeled amino acids, not differing statistically from the control, were observed (Fig. 2). It must be recalled, however, that both zinc and cadmium, if added to the incubation medium, induce synthesis of specific proteins which act as carriers of these trace elements (metallothioneins), in the cell. However, whereas with the concentration of zinc used this synthesis was on a quite considerable scale, in the presence of an MAC of cadmium, the contribution of synthesis of metallothioneins to the total cell protein content is not significant. Cobalt, in a concentration of 0.9 μg/ml, increases incorporation of labeled precursors into RNA and proteins in bone marrow cells in culture [12], which is in contrast with our own data obtained on HeLa cells.

Cytophotometric study of rRNA showed that during incubation with all the trace elements studied the concentration of this metabolite increased toward the end of the day (Fig. 3a). Just as with [$^3$H]uridine, the greatest changes were observed toward this time after addition of cobalt and cadmium. However, addition of this last trace element, especially in the early stages, was accompanied by a decrease in the rRNA content. The characteristic action on synthesis of this same type of RNA also was observed in other investigations [6]. The marked increase in the rRNA content in the cells after addition of nickel is evidently connected with the specific property of this trace element of becoming concentrated in the cell nucleolus [16], and somehow or other of facilitating the synthesis of this metabolite.

The total protein content in the cells showed no significant change as a result of addition of cobalt, cadmium, and fluorine to the incubation medium (fluctuations of not more than 20% from the control level were observed on average). It is known [11] that the protein concentration falls by between 10 and 46% in various organs of rabbits during chronic administration of 50 mg/kg NaF to the animals. The relative stability of the total protein content under the influence of fluorine in the present experiments, on the basis of relatively low incorporation of [$^3$H]leucine is evidently connected with the ability of fluorine to inhibit degradation of various classes of proteins by a greater degree in cell culture [13]. When nickel was used the increase in the protein content in the cells could be associated with elevation of the rRNA level. A decrease in the total protein content under the influence of zinc, especially against a background of relatively high [$^3$H]leucine incorporation, was evidently connected with predominance of catabolic over synthetic processes when the zinc was used in this concentration (Fig. 3b). Loss of total protein also was observed [14] in the kidneys of rats kept on a diet with autoradiographic and cytophotometric data thus showing that cell metabolism in culture is relatively "conservative" in the presence of MAC of cobalt and cadmium. Incubation of cells with MAC of zinc, nickel, and fluorine was accompanied by significant changes in metabolism over a longer period of time. This is rather surprising, for the use of MAC, i.e., concentrations on the threshold of toxic action, ought to lead to short-term and unsubstantial metabolic changes in the cells. The results confirm that these concentrations are not indifferent for metabolism, and they are evidence of the need to take these findings into account when the effects of MAC of certain trace elements on cells are evaluated.

## LITERATURE CITED


1. L. S. Strochkova, A. A. Zhavoronkov, and A. P. Avtsyn, Tsitologiya, 26, 299 (1984).
2. L. S. Strochkova and V. I. Sorokovoi, Usp. Sovrem. Biol., 96, No. 2(5), 211 (1983).
3. M. P. Chekunova, N. A. Minkina, and I. M. Suvorov, Gig. Truda, No. 12, 50 (1978).
4. M. Ando, Environ. Res., 27, 446 (1982).
5. D. Beach and F. Sunderman, Cancer Res., 30, 48 (1970).
6. J. Cervera, M. Alamar, A. Martinez, et al., J. Ultrastruct. Res., 82, 241 (1983).
7. K. Gallagher and I. Gray, Fed. Proc., 39, 2021 (1980).
8. G. Hilderbrand, B. Griffith, and M. Enger, Fed. Proc., 39, 2018 (1980).
9. R. Holland, Acta Pharmacol., 45, 96 (1979).
10. J. Hsu, in: Zinc and Copper in Clinical Medicine, Vol. 2, New York (1978), pp. 25-34.
11. A Kathpalia and A. Susheela, Fluoride, 11, 125 (1978).
12. T. Niebroj, Folia Haemat., 86, 293 (1966).
13. B. Poole and M. Wibo, J. Biol. Chem., 248, 6221 (1973).
14. S. Eana, V. Agrawal, and N. Bhardwaj, Arh. Hig. Rada Toksikol., 32, 157 (1981).
15. B. Vallee and K. Fulchuk, Phil. Trans. Roy. Soc. London, B294, 185 (1981).
16. M. Webb and S. M. Weinzierl, Brit. J. Cancer, 26, 292 (1972).