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RNA SYNTHESIS IN ORGANS OF MICE WITH CHRONIC FLUORINE POISONING

A. P. Avtsyn,* A. A. Zhavoronkov, and L. S. Strochkova

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KEY WORDS: RNA synthesis; experimental fluorosis; fluorine.

A prolonged excessive intake of fluorine causes a severe illness known as fluorosis, which is characterized not only by specific changes in the bony skeleton and teeth, but also by destructive changes in other internal organs. Fluorosis is thus a systemic disease affecting the body as a whole [1]. This suggests that the morphological changes in fluorosis are preceded by injury to metabolic stages common to all cells.

For these reasons, in the investigation described below, changes in RNA synthesis were studied in different organs of mice with experimental fluorosis.

EXPERIMENTAL METHOD

Sodium fluoride was injected subcutaneously in a dose of 12 μg/g body weight daily into male CBA mice weighing 18-20 g in two series of experiments. Animals of the control group received the corresponding volume of physiological saline. The mice were decapitated 1, 2, 3, and 4 weeks after the beginning of the experiment. An intraperitoneal injection of ^{3}H-uridine in a dose of 10 μCi/g body weight, with a specific activity of 10 Ci/mole, was given to the animals 1 h before sacrifice, so that the intensity of synthesis of high-turnover RNA in the cells could be determined. Pieces of various organs were excised from the animals soon after decapitation, washed in Hanks' solution, and placed in concentrated formic acid in the proportion of 1 ml acid to 20 mg tissue. After hydrolysis of the tissue (12 h at 37°C) the samples were examined on a liquid scintillation counter. The results were subjected to statistical analysis on a Nairi computer.

*Academician of the Academy of Medical Sciences of the USSR.

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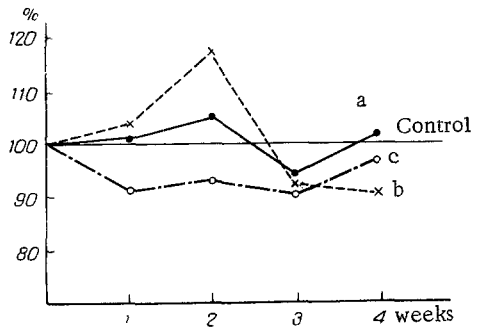

Fig. 1

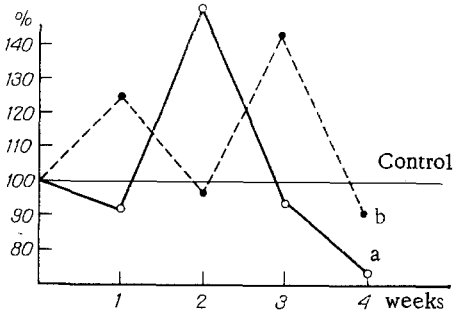

Fig. 2

Fig. 1. Dynamics of RNA synthesis in brain stem and cerebellum (a), cerebral hemispheres (b), and testes (c) during development of fluorosis, Abscissa, duration of fluorine poisoning (in weeks); ordinate, intensity of incorporation of ^{3}H-uridine (in % of control).

Fig. 2. Fluctuations in incorporation of labeled RNA precursor into large (a) and small (b) intestine during fluorine poisoning. Abscissa, duration of development of fluorosis (in weeks); ordinate, quantity of radioactive ^{3}H-uridine in RNA (in % of control).

EXPERIMENTAL RESULTS

The organs of the mice were distributed into three groups on the basis of the results of incorporation of ^{3}H-uridine, depending on the intensity of RNA synthesis in fluorine poisoning. For instance, no significant changes in RNA metabolism were found (Fig. 1) in certain organs (testis, various parts of the brain). Organs such as the small and large intestine exhibited a paradoxical response to administration of fluorine in the form of fluctuations in synthetic activity with time (Fig. 2). Most of the organs studied, however, were characterized by a decrease in the intensity of incorporation of ^{3}H-uridine and, consequently, of RNA synthesis compared with the control ($P < 0.05$). In some organs of this group (heart, liver, tissues of the knee joint), moreover, a sharp fall in RNA transcription was observed as early as during the first weeks of fluorine poisoning, and in the subsequent period it varied very little (Fig. 3, I). A sharp fall in incorporation of the isotope in the first stages of the experiment followed by an increase in synthetic activity almost up to the control level during continued administration of fluorine was observed in the adrenals, spleen, and muscle tissue (the quadriceps femoris muscle) (Fig. 3, II). In some organs (lungs, kidneys, pancreas, duodenum) there was a gradual fall in RNA synthesis, which was most marked at the end of the period of exposure — by the 3rd or 4th week of fluorine poisoning (Fig. 3, III).

The results confirm the view that the metabolic disturbances in different tissues of animals differ in character during the development of fluorosis, partly because of differences in the distribution of this trace element in the body, in the dynamics of its penetration and accumulation, and in its excretion from individual organs. Since organs differ sharply in their enzyme spectrum, both qualitatively and quantitatively, this also represents a wide choice for fluorine, an enzyme inhibitor, in its action on the various aspects of cell metabolism. In most cases this effect of fluoride is known to be connected with its ability to form a weakly dissociating complex with magnesium ions [8, 10]. As a result, the activity of enzymes utilizing magnesium ions as a coenzyme is sharply reduced. The degree of inhibition under these circumstances depends functionally on the quantity of magnesium ions required by a particular enzyme and on the extent to which magnesium can be replaced by other activators. This group includes, for example, several enzymes (5-nucleotidase, adenosine deaminase) catalyzing individual stages of biosynthesis of nucleotides and nucleic acids [6, 9, 13]. As a result, during fluoridation, as has been found in plant cells, the pool of free nucleotides (especially triphosphate nucleotides) is increased, their utilization for synthesis is reduced, and the ratio of the nitrogenous bases in RNA is changed, and this may perhaps reflect variation in the types of RNA synthesized [6]. Some enzymes maintaining cell respiration (glycolysis: enolase, phosphoglucomutase, phosphoglycerate kinase; the tricarboxylic acid cycle: isocitrate and succinate dehydrogenases) are inactivated in the same way on administration of fluoride [4, 10, 12]. The degree to which fluoride forms complexes with magnesium ions may vary in different compartments of the cell, and this may lead to local changes

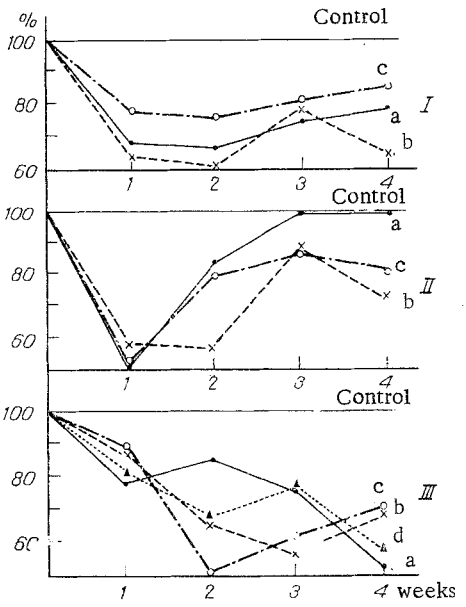

Fig. 3. Decrease in intensity of RNA synthesis in some organs of mice with experimental fluorosis. I: a) heart, b) liver, c) knee joint tissues; II: a) quadriceps femoris muscle, b) adrenals, c) spleen; III: a) lung, b) kidney, c) pancreas, d) duodenum. Abscissa, duration of administration of sodium fluoride (in weeks); ordinate, level of incorporation of ^{3}H-uridine (in % of control).

in concentration of the activator. In turn, this may affect other Mg-dependent functional indices of the cell — functioning of the RNA-polymerase system, the free energy of ATP hydrolysis, the conformational structure of some types of RNA molecules, and, consequently, their functional activity in different parts of the cell [14]. However, the existence of a wide group of enzymes sensitive to fluoride *in vitro* and functionally unconnected with magnesium ions points to other mechanisms of inhibition also. In addition, the enzyme activity of certain proteins, such as pyruvate kinase, is inhibited by fluoride only *in vivo* and not *in vitro* [12]. In the opinion of the authors cited, this is due to suppression of synthesis of the precursors and mediators for these enzymes as a result of the primary action of fluorine. It has been suggested that the disturbance of the cell energy metabolism as a result of suppression of synthesis of ATP and utilization of its reserves because of inhibition of ATPase by fluoride [2, 3, 13] is the true cause of depression of the synthetic activity of the cell in fluorine poisoning.

Fluctuations in RNA synthesis observed in certain organs may perhaps, on the one hand, be connected with the ability of these organs to break the "vicious circle" of inhibition created by fluorine (for example, on account of reserves of enzymes). On the other hand, the possibility cannot be ruled out that at certain stages of its action, fluorine, by disturbing the integrity of cell membranes [7], leads to activation of the enzyme adenylate cyclase, thus leading to stimulation of certain synthetic processes in the cell.

The absence of changes in metabolism of high-turnover RNA in the brain and testicular tissues is most probably due to the use of an insufficiently sensitive technique for determination of the total intensity of synthesis, for use with such heterogeneous organs rather than to the influence of the blood—brain and blood—testicular barriers.

Depending on the enzymes which are inhibited and on the degree to which the tissue can replenish its "energy reservoir," for example through the pentose shunt, enzymic catalysis in which is to some extent resistant to fluoride [5, 11], qualitative differences are observed in the kinetics of RNA synthesis in the various organs of animals poisoned with an excess of fluoride.

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TOXICITY OF HIGH-MOLECULAR-WEIGHT PROTEIN FROM BURNED SKIN REVEALED BY BIOLOGICAL TESTS *in vivo* AND *in vitro*

B. E. Movshev, R. V. Nedoshivina, and I. K. Koryakina

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KEY WORDS: burns; toxin; methods of determination.

The study of burn toxemia — an important stage in the pathogenesis of burns — has for a long time been hindered by the lack of adequate methods for the isolation of toxic substances. Attempts to purify burn toxin have frequently been carried out and have been partly successful [13, 14]. Contrary to expectation, however, investigations of "Rosenthal's glycopeptide" and "Schoenenberger's lipoprotein" have not been pursued further, possibly because the methods used to isolate the toxins were laborious and, more important, nonspecific.

In the researches of Fedorov and co-workers attention was concentrated on the immunologic specificity of factors of burn toxemia [1, 3, 5, 7, 10, 11]. A detailed study of products from burned skin served as the basis for development of a direct immunochemical method of isolation of the toxin [12], a high-molecular-weight protein toxin was isolated for the first time [6], and its high pathogenicity was demonstrated [8].

The object of this investigation was to determine the comparative characteristics of activity of the high-molecular-weight protein toxin of burned skin on the basis of the results of biological testing by two independent methods, used in the writers' laboratory to study burn toxemia.

EXPERIMENTAL METHOD

The skin of 207 burned Wistar rats was used as the source of the toxic material. A burn affecting 15-20% of the body surface was inflicted by the flame of a cotton swab soaked in alcohol for an exposure of 45 sec. Extracts of burned skin (EBS) were prepared by the method in [4].

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