FLUOROSIS: GEOGRAPHICAL PATHOLOGY AND SOME EXPERIMENTAL FINDINGS

by

A.A. Zahvoronkov, and L.S. Strochkova
Moscow, USSR

Endemic fluorosis is related to a high concentration of fluoride in water. Such springs are found in regions of disintegrating granite, in regions of former or current volcanic activity and in natural phosphate zones. A certain role in the development of endemic fluorosis is also attributable to the consumption of food grown on soils rich in fluoride.

The actual prevalence of endemic and industrial fluorosis is unknown. However a search of the relevant literature covering 15 years allows one to conclude that not less than 20 million people in the world are affected by this disease. Areas of endemic fluorosis occur in all inhabited continents (Fig. 1). They are being studied most extensively in North

Figure 1

Geographical Distribution of Endemic Fluorosis Throughout the World

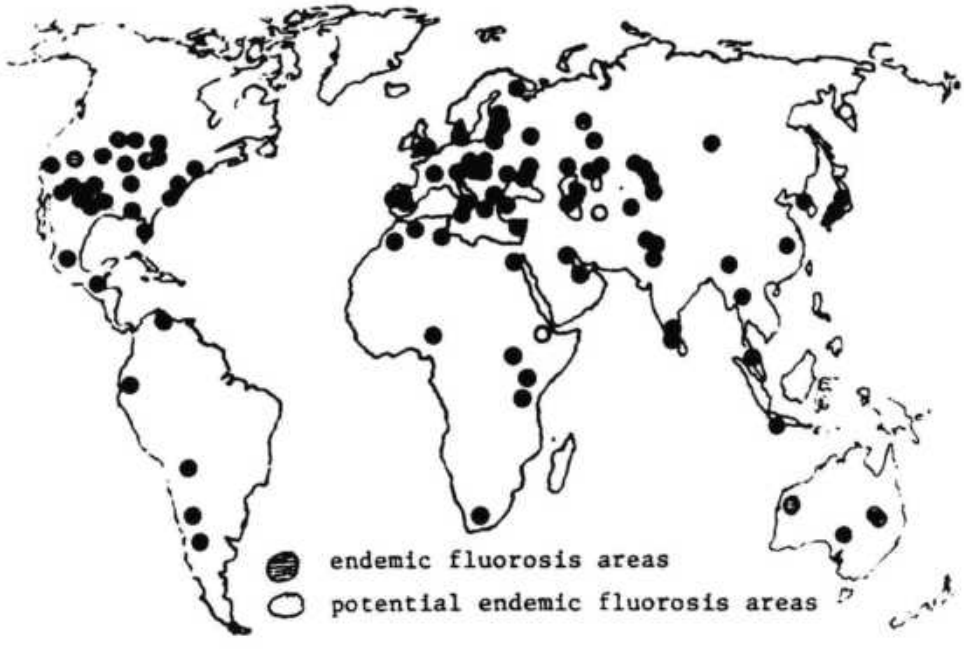

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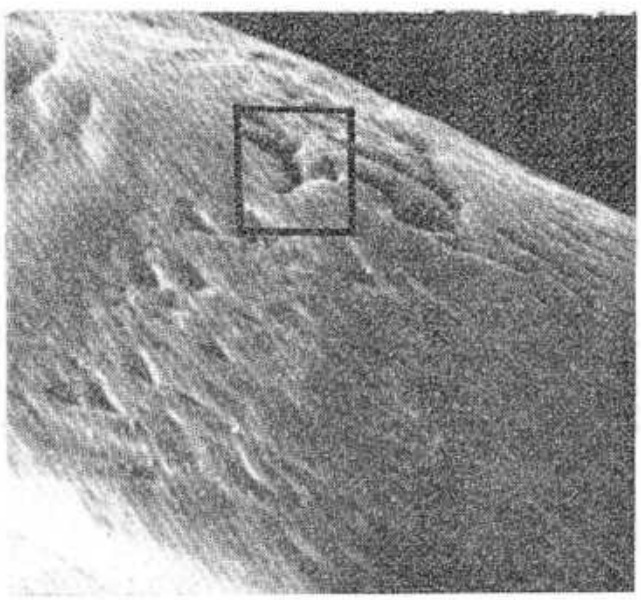
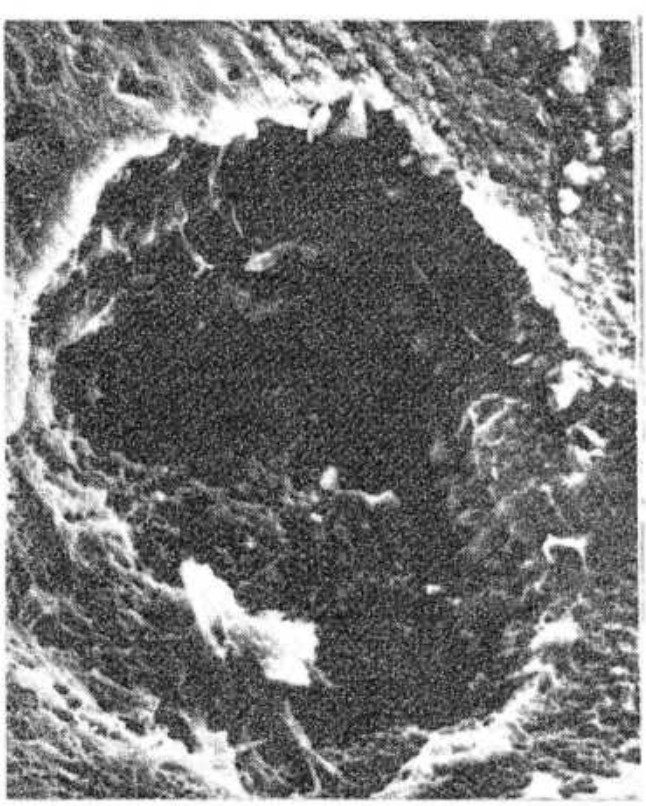
Figure 2

Enamel Changes of Rat Incisors in Experimental Fluorosis



a. Enamel showing numerous fissures and erosions (x40).

b. Same (x400).



c. Deep erosion of enamel(x800).

d. Eroded enamel surface "cobblestone road" (x210).

America (USA, Canada), Europe and those Asiatic countries where the problem is widespread as, for example in India. In the last-mentioned, the zone of fluorosis is designated "fluorosis belt."

In the Northern European part of the Soviet Union, in Siberia and in the Far East, the drinking water is low in fluoride. The vast majority of the world population consumes water low in fluoride (less than 0.5 ppm).

An extensive survey of an area of endemic fluorosis in the North Kazakhstan region of the Soviet Union revealed that the fluoride content of rocks was twice as high as that in clay. The concentration of fluoride in lake water was as high as 11 ppm and in drinking water, 4 ppm. The maximal concentration of fluoride in milk was 0.5 ppm. In locally grown cabbage, the fluoride content was up to 3 ppm. The maximal concentration of fluoride in human blood was 0.62 ppm, in the teeth, 776 ppm. In hair the maximal level is 72 ppm which is 10 times lower than in a nonendemic area.

In children born in the endemic area, consuming water with 4 ppm fluoride, dental fluorosis occurred in 91.8%, and dental caries in 40.7%. In children who came to the endemic area after birth, dental fluorosis was recorded in 47.2% and caries in 55.8%. Adults were affected by fluorosis in 46.8%, by caries in 72.4% and by a combination of both (fluorosis and caries) in 31.4%.

Experiments on white rats receiving daily 12 mg of sodium fluoride per kilogram body weight revealed specific changes of the incisor enamel pigmentation. The scanning electron microscopic study of the incisors showed pronounced alteration of the enamel in the form of irregular structure of enamel prisms and the presence of fissures and erosions. The normal prismatic structure of the enamel was replaced by a globular structure and the surface of enamel resembled a "cobblestone road" (Fig. 2).

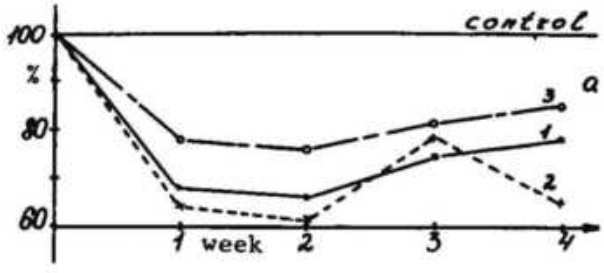
Next we studied the dynamics of RNA and protein synthesis in some organs of CBA-mice. The animals were decapitated 1, 2, 3, and 4 weeks after daily subcutaneous injections of 12 micrograms of sodium fluoride per gram of bodyweight. One hour before slaughter, the animals received intraperitonealy ^{3}H-uridine and ^{3}H-leucine. The scintillation was performed after dissolving the pieces of tissues in concentrated formic acid and the data obtained were processed on a computer.

The studied organs were classified into three groups according to the intensity of rapidly tracing RNA synthesis. In testicles and in different parts of the brain no significant changes of the RNA metabolism were revealed. In the ileum and rectum the synthetic activity broadly varied in time. In such organs as the heart, liver, and knee joint a marked decrease of RNA transcriptions was recorded as early as during the first week of intoxication (Fig. 3a). In adrenals, in the spleen and in the muscles, a marked decrease in isotope insertion was revealed at the first stages of the experiment. Later, in spite of continuing intoxication (Fig. 3b), it was replaced by increased synthetic activity almost to the control level. In the lungs, kidneys, pancreas, and duo-

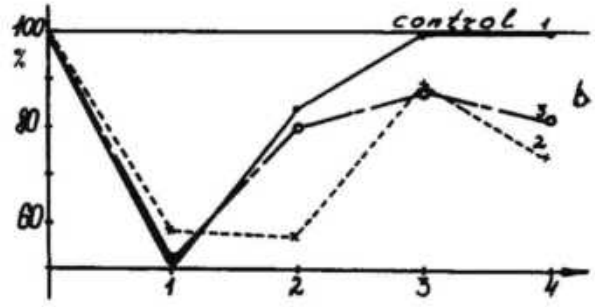
denum, a gradual decrease of RNA synthesis took place which reached its maximum at the termination of the experiment (Fig. 3c).

Figure 3

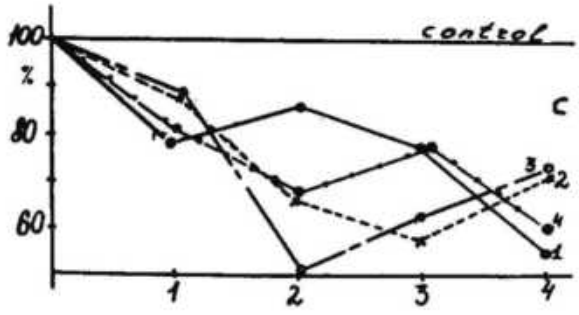
Decrease of RNA Synthesis in Mouse Organs During NaF Intoxication (% of control)



a. 1-heart, 2-liver, 3-tissues of knee joint.



b. 1-muscle tissue, 2-adrenal gland, 3-spleen.



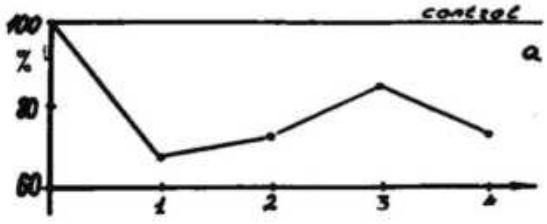
c. 1-lung, 2-kidney, 3-pancreas, 4-duodenum.

Fluoride intoxication had a different effect also on protein synthesis which accounted for a classification of the mouse organs into three groups. In different parts of the brain, in testicles, and in the ileum, the fluctuations in the isotope did not differ significantly from the control values. In the spleen, adrenals, liver and duodenum pronounced fluctuations of protein biosynthesis were directly correlated with the increase in fluoride levels. However in most of the other organs, the development of fluorosis was accompanied by a marked decrease of the protein synthesis in comparison with the control level. Whereas this decrease became manifest in the tissues of the knee joint during the first weeks of the experiment (Fig. 4a), in muscle tissue and in the colon inhibition of protein synthesis occurred only after 3 - 4 weeks of fluoride administration (Fig. 4b). In the kidney and the pancreas,

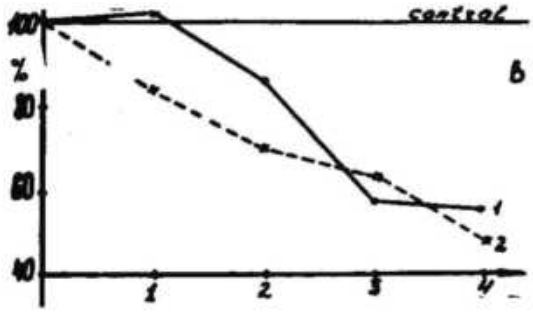
the inhibition of the precursor insertion in the beginning of the experiment was replaced later by an increase of the synthetic activity almost to the control level. In the heart and the lungs, the intensity of protein synthesis decreased in the middle of the experiment (Fig. 4c, d).

Figure 4

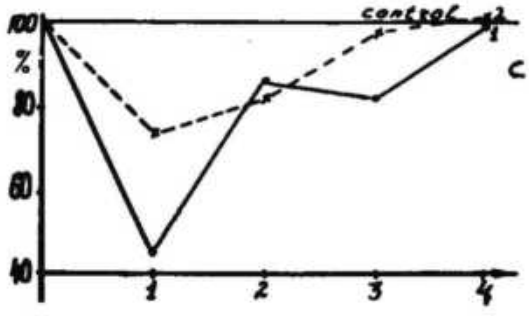
Inhibition of Protein Synthesis During NaF Intoxication



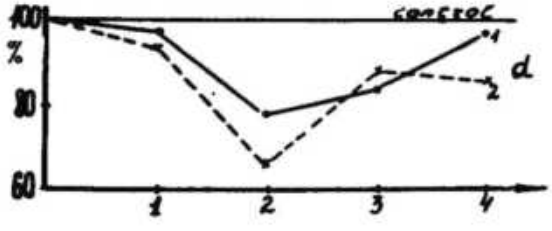
a. knee joint



b. 1-muscle, 2-rectum



c. 1-kidney, 2-pancreas



d. 1-heart, 2-lung

The depression of protein synthesis is probably related to a decrease in the RNA transcription, although a strict parallelism between these two processes in most of the studied organs was not established. The disturbance of the protein-synthetizing system in fluorosis also is attributable to a decrease in activity of a group of enzymes, catalyzing the key processes of cellular metabolism. The group includes enzymes catalyzing certain stages of biosynthesis of nucleotides and nucleic acids (5^1-nucleotidase, adenosidedesaminase), enzymes catalyzing certain stages of

aminoacid synthesis (glutaminesynthetase) and enzymes catalyzing certain stages of protein synthesis (methionin-activating enzyme of the liver).

Administration of sodium fluoride inactivates also certain enzymes of glycolysis and of the tricarboxilic acid cycle. The action of fluoride on these enzymes, which in most cases are magnesium-dependent, is related to production of weakly dissociating complexes of magnesium-fluoride and enzymes. Consequently the activity of these enzymes, in which magnesium is a cofactor, sharply declines.

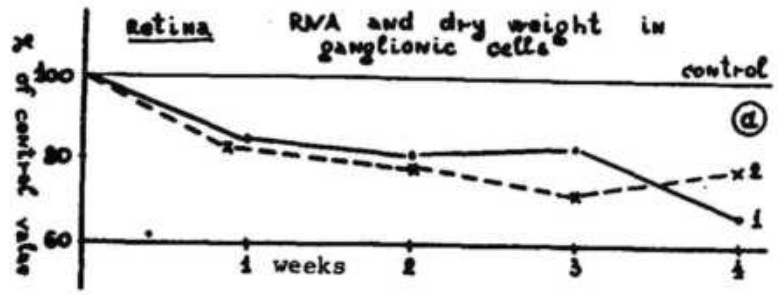
The degree to which fluoride complexes with magnesium ions can vary in different cell compartments causing local changes in the concentration of the activator. This in turn affects the function of the RNA-polimerase system, the free energy of ATP hydrolysis and the conformational structure of some types of RNA and proteins.

In the same series of experiments, the metabolic changes in certain organs of the mouse were studied by morphological methods. In the ganglion cells of the retina, the content of rRNA was estimated by the cytophotometric method and the dry weight was calculated by means of interpherometry. Administration of fluoride produced a progressive decline in the rRNA content and in the dry weight (Fig. 5a). By means of the use of ^{3}H-uridine and ^{3}H-leucine this event was found to be associated with a decrease of synthetic processes in such types of cells. A similar decrease in precursor insertion was revealed by autoradiography also in the other layers of the retina. The inhibition of RNA synthesis was more pronounced in the ganglion cells and the decrease of protein synthesis in perikaryons of the photoreceptors (Fig. 5b, c). During the course of the development of pathologic changes in the blood vessels of the retina, the penetration of fluoride through the hemato-ophthalmic barrier increases (3). This in turn intensifies the process of inhibition in accordance with the "cascade" principle. A hypothesis is suggested correlating the disturbance of the ascorbic acid metabolism and the development of fluoride retinopathy in fluorosis. It is known that fluoride can inhibit the action of certain enzymes in the epithelium of the ciliary body which are essential for the transport of the oxidized form of ascorbic acid into the eye. Fluoride also changes the metabolism of the ascorbic acid in the body. The "vicious circle" in the ascorbic acid metabolism by the feed-back mechanism increases necrotic processes in the retina. Thus this vitamin may be indicated as a prophylactic remedy in fluorosis.

A.P. Tarinsky (4) revealed a 2-3 fold increase of symptoms of oligospermia and azoospermia in male workers suffering from industrial fluorosis compared with healthy men of the same age. Tokar (5, 6) found an association between fluorosis and hypogonadism. These data made it necessary to study the changes of synthetic processes in the testes of mice in experimental fluorosis. A total scintillation method of probe computing on the counter revealed certain shifts of RNA and protein metabolism in this organ, but the data were not statistically significant. More informative, was the cytochemical investigation of certain cell types in the testes on the separate stages of spermatogenesis, particu-

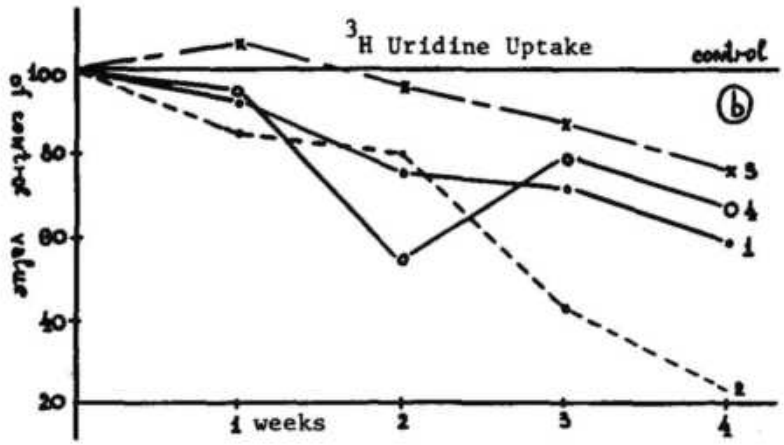
Figure 5

Metabolic Changes in Mice Retina During NaF Intoxication

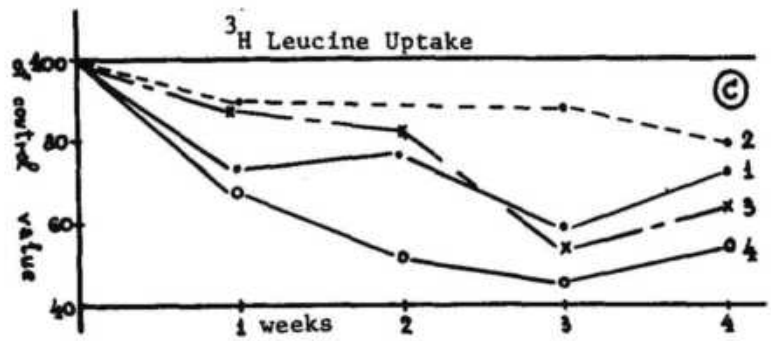


a. Progressive decrease of RNA content (1) and of dry weight (2) of ganglion cells.

RNA Synthesis in Different Layers of Retina



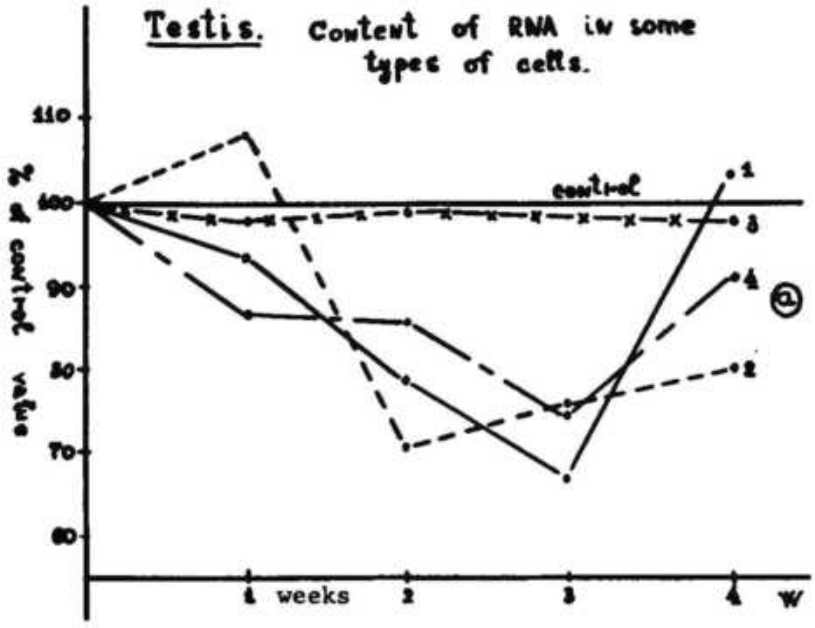
Protein Synthesis in Different Layers of Retina



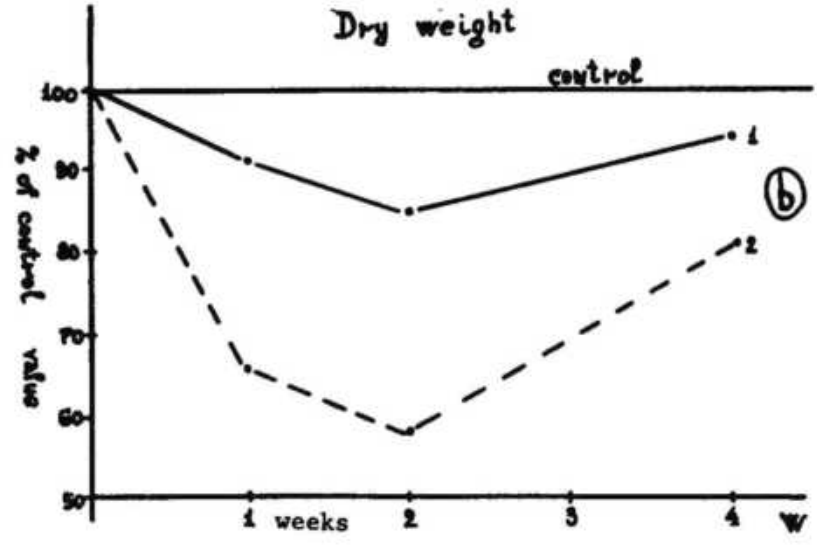
b. and c. 1-total uptake of H uridine in the retina, 2-ganglionary cells, 3-photoreceptor perikaryons, 4-bipolar perikaryons.

Figure 6

Metabolis Changes of RNA and Protein in Different Cells of Mice Testes During NaF Intoxication



a. 1-Leidig cells, 2-Sertoli cells, 3-spermatogones A, 4-spermatides.



b. Decrease of dry weight of spermatides (1) and of Leidig cells (2) on the 7th stage of spermatogenesis.

larly on the 7th stage. The cells of spermatogenic epithelium with fairly stable DNA content - Sertoli cells, spermatogones of A type, spermatides and also interstitial cells of Leidig - were studied. Between the second and third week after the beginning of the experiment there was a decrease of rRNA in the basal part of the Sertoli cells, in Leidig cells and in spermatides.

In the spermatogones this index was not significantly changed (Fig. 6a). During the course of hyperfluoridation a decrease of the dry weight appeared in Leidig cells and in spermatides (Fig. 6b). An adaptation of the cells to the toxic influence of fluoride is suggested since at the termination of the experiment a tendency to normalization of the rRNA content developed and the dry weight of the spermatides and the interstitial cells reached almost the control level. It is known that Sertoli cells constitute a part of the hematotesticular barrier and are actively attacked by fluoride ions, which is perhaps the cause of the decrease of rRNA in these cells. On the other hand, these cells play a trophic role for spermatides and probably supply them with rRNA. Consequently the decrease of the rRNA content and of the dry weight in spermatides is caused by the disturbance of the metabolism in Sertoli cells and therefore presents a secondary event.

The noted cytochemical alterations in Leidig cells and in the basal parts of Sertoli cells reflect the disturbances in the protein synthesizing system of these cells in fluorosis and to a certain degree explain the hormonal imbalance in this disease, since Leidig cells synthesize testosterone and Sertoli cells produce protein-binding androgens. The high resistance of the spermatogones to the influence of fluoride in comparison with Sertoli cells is difficult to explain since in most cases the unfavorable factors affect primarily the sperm cells.

Since the various stages of spermatogenesis are controlled by different hormones (e.g. testosterone controls the process of meiosis) (2) the present findings allow one to develop a more complete concept of the alteration of the germinative epithelium in fluorosis - not only through the disturbance of the functioning of the cell enzyme systems but also by the way of induction of hormonal imbalance in the body.

In conclusion we can state that fluorosis affects the whole organism with elective lesions of the teeth and the skeleton. The pathogenesis of fluorosis in many aspects remains unclear. Intrinsic mechanisms of the influence of fluoride on the body take place on the cellular and molecular levels as illustrated in this report by demonstration of certain cellular alterations and disturbances of systems in fluorosis from fluoride in water naturally and in experimental fluorosis.

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