HISTOMORPHOLOGICAL CHARACTERISTICS OF SOME ENDOCRINE GLANDS OF RATS POISONED WITH FLUORINE

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UDC 615.916:546.16].092.07:
616.43-091.8-07

An experimental model of fluorosis obtained in rats by daily subcutaneous injection of sodium fluoride solution in doses of 0.03, 0.1, and 1.2 mg/100 g body weight for 206 days was used to provide material for studying the thyroid and parathyroid glands. The experiments showed that fluorine has a slight thyrostatic effect. Hyperplasia of the pale cells of the thyroid and of the epithelial cells of the parathyroid gland was observed.

The intimate mechanisms of fluorine poisoning, especially those associated with activity of the endocrine glands, have been inadequately discussed in the literature and require further study [2-4, 7, 9]. The question of whether the toxic action of fluorine on the organs and tissues is direct or indirect, through a disturbance of the functions of the endocrine glands, especially the thyroid and parathyroid glands, still remains unanswered.

It was therefore decided to study the effect of fluorine on the development of changes in these glands.

EXPERIMENTAL METHOD

Sodium fluoride solution in doses of 0.03, 0.1, and 1.2 mg/100 g body weight was injected subcutaneously, daily, into 179 albino rats of both sexes weighing initially 100 ± 20 g. The first two doses were given for 206 days (1st and 2nd groups), the third for 60 days (3rd group). The control group consisted of 26 rats kept under the same conditions but not receiving sodium fluoride. Organs from the animals of the first two groups were investigated after sacrifice on the 33rd, 69th, 103rd, 131st, 166th, and 206th day of poisoning, and those of the third group on the 11th, 28th, and 56th days. In the after-period of fluorine poisoning the investigations were carried out on the 9th, 29th, 66th, and 135th days and on the 7th and 123rd days respectively for the animals of these groups.

Fluorosis of the teeth of the rats of the first two groups was observed 2-2.5 months after the beginning of the experiment, while in a dose of 1.2 mg these times were shortened to 2 weeks. For a more objective evaluation of the changes which were discovered in the thyroid gland, the following parameters were determined: 1) the diameter of 30 follicles; 2) the height of the follicular epithelium; 3) Braun's index (the ratio between the diameter of the follicle and height of the thyroid epithelium); 4) the number of pale parafollicular cells per 10 fields of vision. In the parathyroid gland the area of the cell and nucleus was determined. The results were analyzed by statistical methods.

EXPERIMENTAL RESULTS

Under the influence of low and, in particular, of high doses of sodium fluoride large areas of hyperplasia, accompanied by degenerative changes, were observed in the thyroid. Proliferation affected mainly the interfollicular cells. Only the differences in height of the follicular epithelium in the period of poison-

Research Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Pvtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 69, No. 6, pp. 107-110, June, 1970. Original article submitted November 15, 1969.

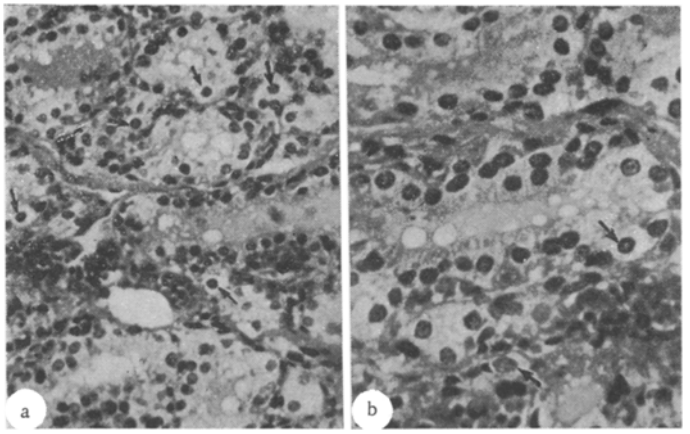

Fig. 1. Thyroid gland. Arrows point to pale cells. a) Parafollicular pale cells (hematoxylin-eosin, 250 ×); b) the same (500 ×).

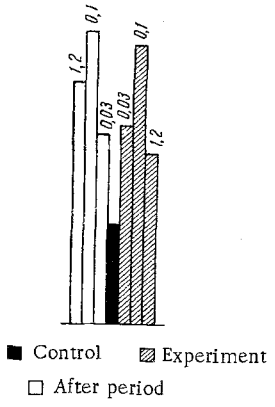

Fig. 2

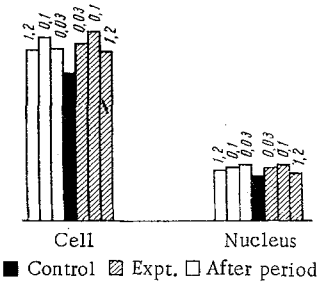

Fig. 3

Fig. 2. Diagram showing mean indices for number of pale cells in all groups of the experiment.

Fig. 3. Diagram showing mean indices of area of cytoplasm and nucleus of epithelial cells of the parathyroid gland in all groups of the experiment.

ing were significant ($t > 2$; $P < 0.05$) in the animals of the first two groups, when poisoning was of long duration (up to 206 days). Braun's index also was increased in these experimental groups, but the difference was not statistically significant. However, this could still be evidence of a definite tendency for thyroid function to be depressed.

Meanwhile changes were discovered in the pale parafollicular cells of the thyroid. These cells were observed in the wall of the follicles, in the interfollicular pads, and in the zone of contact between follicles. They were nonpolar, had no contact with colloid, were arranged in parafollicular groups of 2-3 or, rarely, 3-5 cells, and as a rule they had direct contact with the capillary wall. Their nuclei were large and round. The mean diameter of the pale cell and its nucleus was 17 and 7 μ, and their area 221 and 44 μ^2. The corresponding figures for the follicular cells were 11 and 6 μ, and 80 and 33 μ^2 (for material fixed in Carnoy's solution). The cytoplasm of the pale cells was poorly stained by acid dyes (Fig. 1).

Mean values for the number of pale cells in all groups of the experiment are shown in the diagram (Fig. 2). A definite relationship was established between the increase in number of pale cells and the dose and duration of poisoning. The most demonstrative increase in their number was observed in the first two groups of the experiment, and this also was evidently related to the duration of poisoning. The sharp increase in the number of pale cells observed in the experimental rats compared with the controls was statistically significant ($t > 3$; $P < 0.05$).

The effect of fluorine on the state of the parathyroid gland was judged from the change in the area of the cytoplasm and nucleus of the epithelial cells. Their mean indices are given in Fig. 3. The diagram shows that the same relationship holds between the area of the epithelial cells and the duration of poisoning. Statistical analysis of the results demonstrates that the differences between experiment and control are significant ($t > 2$; $P < 0.05$), evidence of a strain on parathryoid function.

The mechanism of action of fluorine on the animals organism is complex. Fluorine has a mild thyrostatic effect. The hyperplastic reaction which was observed is a manifestation of the general adaptation-reflex response to lowering of the iodine balance, just as is observed in a focus of epidemic goiter. This state of affairs is in agreement with the previously established fact that fluorine has a direct depressant effect on the absorption of inorganic iodine [10].

In an experiment similar to that now described [7], similar changes in the thyroid were explained by an increase in the secretion of thyrotropic hormone.

Like thyrotropic hormone, fluorine stimulates the oxidation of glucose and phospholipid metabolism in the lobes of the thyroid gland, and also activates adenylcyclase in a thyroid tissue homogenate. However, unlike thyrotropic hormone, fluorine does not cause the intracellular formation of colloid droplets, as has been demonstrated previously [13].

Changes in the roentgenologic picture of the skeleton found in the experimental animals are evidence of a disturbance of calcium and phosphorus metabolism in chronic fluorine poisoning. This metabolism is regulated by two hormones: parathormone secreted by the parathyroid and thyrocalcitonin secreted by the pale cells of the thyroid [11]. However, the existence of yet another hypocalcemic hormone, with a different molecular weight and amino-acid composition, has now been reported [6].

The presence of similar resorption cavities in osteitis fibrosa cystica produced by hyperactivity of the parathyroid glands and by skeletal fluorosis prompted [8] an electron-microscopic investigation of the parathyroid glands of sheep with fluorosis, with the simultaneous determination of the blood level of parathormone. This investigation showed that the parathyroid gland is hyperactive in fluorosis.

In the light of the new data concerning the active participation of the pale parafollicular cells of the thyroid gland in chronic fluorine poisoning obtained in the present investigation, the pathogenic link between changes in the studied glands can be represented as follows. Hyperplasia of the parathyroid gland with hyperproduction (5 times more than normally) of parathormone [8] activates the compensatory and adaptative mechanisms of the body. In particular, the pale cells of the thyroid, which secrete thyrocalcitonin, an antagonist of parathormone, undergo hyperplasia. At a certain stage this mechanism balances the processes of elimination of calcium from the bones and its deposition in them. This explanation of the action of fluorine is also supported by numerous facts indicating that the serum calcium level is unchanged in both man and animals with fluorosis [1, 5, 8].

In this connection the writers consider that in skeletal fluorsis new bone tissues is formed, not through the stimulant effect of parathormone [12, 9], but through the influence of thyrocalcitonin. It has also been postulated [12] that fluorine induces a special form of secondary hyperparathyroidism. This hypothesis was confirmed by the results of the present experiments on chronic fluorine poisoning.

Experimental fluorosis in rats is thus accompanied by a secondary hyperparathyroidism and by an increase in the number of specific pale cells in the thyroid, secreting thyrocalcitonin.

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