

# LEUKOCYTE RESPONSE IN YOUNG MICE CHRONICALLY EXPOSED TO FLUORIDE

by

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**SUMMARY:** The study, by light and fluorescent microscopy, of sternal and femoral bone marrow taken from young Swiss mice exposed for periods up to 280 days to elevated levels of sodium fluoride in drinking water, has revealed morphologic abnormalities in cell structure and mitotic figure formation in immature leukocytes. Alterations in the content and distribution of RNA and DNA also appear after several weeks of exposure. These findings, interpreted in relation to other reported data, bear compatibility with a possible shift of these cells toward anaplasia.

## Introduction

The widespread utilization of fluoride as a dental protective measure has raised serious questions concerning its potential hazard to humans. It has been established that repeated exposure to fluoride induces inhibition in the mineralization of bone (osteomalacia), often accompanied by increased osteoclastic activity and osteoid formation (1). Belanger and co-workers (2) have reported the suppression of calcium absorption through the intestinal wall by fluoride-treated suckling pigs. Other investigators (3, 4) have described experimental settings in which fluoride stimulated the formation of new bone.

The chief cells in the parathyroid glands of rats, chronically exposed to fluoride, undergo an increase in metabolic activity (5). Similar observations have been made in the human parathyroid glands (6). The effects of fluoride upon parenchymous organs have proved to be something less than dramatic. The only positive change found in the liver and kidneys of rats after prolonged fluoride exposure, is a rise in lipid and glycogen deposition (7,8).<sup>\*</sup> Recently alterations in white blood cells in relation to fluoride treatment have been reported (9-11).

The hematopoetic system is constantly being exposed to the slightest presence of fluoride (12). Changes in mature fluoride-exposed murine leukocytes have been previously documented (13). Therefore it is deemed of special significance to investigate the results of fluoride upon rapidly dividing immature cells.

## Materials and Methods

Young Swiss mice of both sexes were divided into three groups. Twenty animals (group I) received only distilled drinking water containing sodium

\* Editor: See Ramseyer et al., J. of Gerontology, 12:14-19, 1957.

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fluoride at 11 ppm concentration. Twenty mice (group II) received 22 ppm sodium fluoride in distilled drinking water. These fluoride levels were selected because 22 ppm is the previously determined maximum at which these animals could be chronically maintained. Also, this concentration of fluoride bears a relationship to elevated levels sometimes occurring in human infants (14). A control series (group III) of 10 animals was given only distilled water to drink. The mice were sacrificed, by cervical dislocation, at intervals of 1 to 280 days. Tissue blocks were removed from the sternum and femur and placed into either 10% buffered formalin or Zenker's solution for fixation with subsequent decalcification in 5% formic acid. Thin sections (3 $\mu$ ) were prepared from paraffin-embedded material and stained with hematoxylin and eosin, the Phloxine methylene blue method of Mallory, and with the acridine orange technique of Bertalanffy for fluorescent microscopy (15). Differential cell counts were made on the phloxine methylene blue-stained sections.

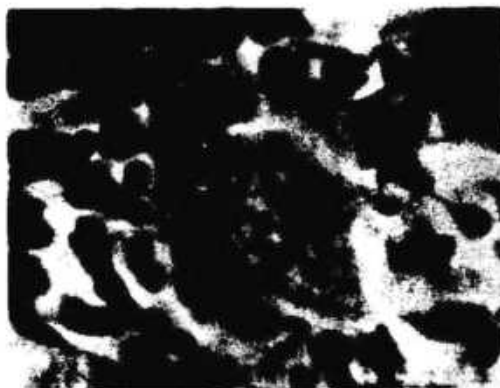
### Results

Recognizable alterations in murine myeloid elements, consequent to fluoride exposure, were observed within 12 weeks. The immature "blast" forms increased in number from an average count of 3 per high power field to a maximum of 25 per high power field at 20 weeks in mice receiving 22 ppm of fluoride.

A change in the normal configuration of mitotic figures was noted after about 8 weeks in mice receiving 22 ppm of fluoride; similar deviations

Figure 1

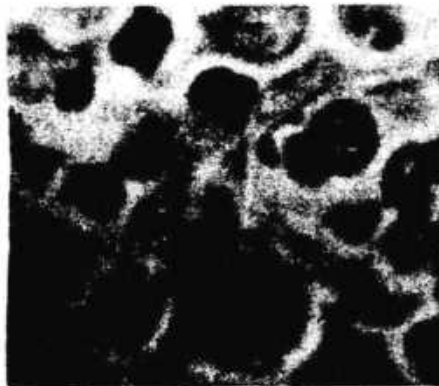
Sternal Bone Marrow Section From  
Mouse Exposed to F<sup>-</sup> for 12 Weeks



Note abnormal nuclear chromatin in a myeloblast at the end of mitosis. H. & E stain x 1200.

Figure 2

Sternal Bone Marrow Section From  
Mouse Exposed to F<sup>-</sup> for 24 Weeks

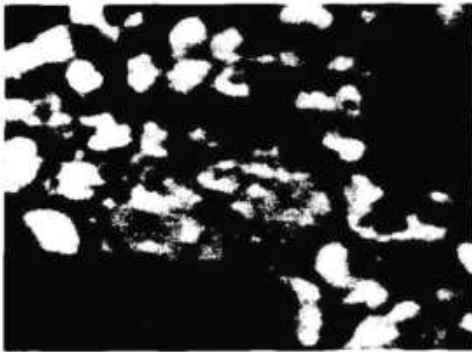


Note abnormal mitotic figure in myelocyte and nuclear chromatin distribution in myeloblast. PMB stain x 800.

were seen at the twelfth week in animals receiving 11 ppm. Both abnormal single polar figures and multipolar mitotic figures appeared (Fig. 1 and 2). The abnormal figures increased after 24 weeks of fluoride exposure, appearing also in myelocytes and metamyelocytes.

Figure 3

Femoral Bone Marrow Section From  
Mouse Exposed to F<sup>-</sup> for 20 weeks



Fluorescent microscopy of acridine orange-stained sections from mice after 12 weeks of fluoride activity (22 ppm) revealed a paucity of cytoplasmic RNA in many "blast" cells. An irregular distribution of RNA was found in the immature cells. The DNA within the nuclei faded as the chromatin network was disrupted (Fig. 3). Uneven clumping of acridine orange-stained RNA was noted in myelocytes and metamyelocytes as the experiment progressed.

Note fragmentation of nucleus of myeloblast and the irregular distribution and clumping of brightly fluorescent RNA in the cytoplasm. Acridine Orange stain x 1200.

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#### Discussion

The increased numbers of "blast" forms observed in the murine bone marrow during the prolonged course of fluoride administration may be understood in the light of previous findings which show that the destruction of peripherally circulating leukocytes stimulates a replacement response by the myeloblastic elements of the bone marrow with an outpouring of immature cells (13, 16,17). The presence of abnormal mitoses in response to fluoride excess in young white cells suggests the presence of a maturation process that has deviated from its usual course and, if continued, will embark upon a pathway toward anaplasia (18,19).

The irregular, punched-out nuclei present in some "blasts", mitotic abnormalities and the irregularity of RNA distribution within the cells

distinguishes them from the giant cells described previously in the bone marrow of patients receiving excessive amounts of fluoride therapy (20).

There seem to be changes in the distribution of RNA within young leukocytes. Fluoride has been found to exert a profound metabolic effect upon leukocytes bringing about an increase in intracellular respiration and glucose oxidation (21). Degranulation of mature leukocytes follows elevations of fluoride concentration within the cytoplasm (11,21).

It is postulated that such degranulation is correlated with the disruption of intracellular metabolism due to excessive fluoride exposure. This element seems to act upon both RNA and DNA content and distribution. Fluoride may have the potential for denaturing these important nucleic acids.

The bright orange fluorescence exhibited by the RNA in the fluoride-treated young leukocytes appears quite similar to the intense coloration described in acridine orange-stained human pulmonary carcinoma cells (22, 23). Under normal circumstances, it has been established that the RNA content decreases as the cells mature (24). Chronic exposure to fluoride in mice may reverse this process so that there is an increased content of RNA that has an irregular cytoplasmic distribution. The nuclear DNA content is also irregular. Evidence that nucleic acids from critical targets for direct acting carcinogens may assist in creating a bridge over the boundary between normal and anaplastic cells (25,26).

A scheme of neoplastic transformation proposed by Miller and Miller (27), provides a plausible explanation for what might be transpiring within the hematopoietic system of fluoride-exposed mice. The key component would be a binding of an ultimate carcinogen, possibly with RNA, within the developing leukocyte, eventuating in its dedifferentiation to a level of anaplasia.

The results of this investigation indicate that young leukocytes chronically exposed to elevated fluoride levels have the potential for an irreversible shift toward the formation of neoplasm.

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