

## MORPHOLOGICAL EFFECTS OF SODIUM FLUORIDE ON HEMATOPOIETIC ORGANS IN MICE

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**SUMMARY:** In connection with earlier studies, we examined early-response morphological effects of sodium fluoride (NaF) on hematopoietic organs in adult mice. Mature Balb C mice were given three injections of NaF in their tail veins on alternate days at total doses of 0, 10, and 50 mg NaF/kg bw. In the mice receiving NaF, morphological abnormalities were seen in the spleen as increased lymphocyte nodules, decreased white pulp, and increased red pulp infiltrated by lymphocytes. The changes were greater at the higher dose and, in other exposed mice, were still evident after three weeks. In contrast to the spleen, the liver, kidneys, and bone marrow showed little alteration.

Keywords: Balb C mice, Bone marrow, Hematopoietic organs, Kidneys, Liver, Sodium fluoride, Spleen, Toxicity.

### INTRODUCTION

Although an association between environmental exposure to fluoride and morbidity from hematological diseases has been reported,<sup>1</sup> and toxic effects of fluoride on various body organs have received considerable attention,<sup>2-4</sup> the potential influence of fluoride on hematopoiesis remains unclear. In his classical studies during the 1930s, Kaj Roholm found a small reduction in the erythrocyte count and slight anemia among cryolite workers.<sup>5</sup> In his animal experiments he encountered definite anemia in calves and dogs from chronic fluoride intoxication.<sup>5</sup> A decade later, Ginn and Volker reported a nearly 30% reduction in blood hemoglobin of an unspecified strain of rats after 86 days with 50 ppm F in their drinking water.<sup>6</sup> In a putative replication of this work, McClure and Kornberg cited contrary findings and found no decrease in the blood hemoglobin of rats.<sup>7</sup> Still later, in 1981, Uslu did not observe anemia in rats after 45 days of exposure to 30 and 100 ppm F in their drinking water.<sup>8</sup>

Other reports are equally conflicting. Hirao *et al* found in rabbits that 2 to 4 weeks exposure to 10 and 50 mg NaF/kg bw led to hypoplasia of bone marrow, markedly decrease total nucleated cell counts and anemia.<sup>9</sup> In 1969 Balazova *et al* reported that children living in industrial regions polluted by fluoride had decreased hemoglobin levels and an increase in the number of erythrocytes in peripheral blood.<sup>10</sup> Uslu, on the other hand, did not find any significant blood changes among either children or adults residing in an area of endemic fluorosis,<sup>8</sup> in agreement with studies by Agate *et al*.<sup>11</sup> According

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to Hoogstratten *et al*, long-term exposure of cattle to 100 ppm F in diet did not cause anemia or detectable abnormalities in bone marrow or liver function.<sup>12</sup> Other studies by Hillman *et al*, however, disclosed anemia as well as hypothyroidism in dairy cattle from excessive ingestion of fluoride.<sup>13</sup>

Since most earlier experimental studies on hematopoiesis focused on effects of fluoride on peripheral blood or bone marrow morphology, we decided to undertake a closer examination of hematopoietic effects of fluoride intoxication. In this regard the present work can be seen as an extension of our previous research in the field of hematopoiesis.<sup>14-16</sup> Here we report an investigation of early response morphological changes in organs that actively participate in hematopoiesis (liver, spleen, kidneys, and bone marrow) in mice injected with sodium fluoride.

#### MATERIALS AND METHODS

*Mice:* The experiment was performed on pathogen-free, 5-week-old, mature female inbred Balb C mice (Polish Academy of Sciences, Wroclaw, Poland). The animals were randomly divided into one control and three experimental groups containing 6 mice each. The animals were maintained under standard laboratory conditions in a 12hr/12hr light-dark cycle at 21°C.

*Injection of mice with NaF:* Sodium fluoride (Sigma, USA) was diluted in a phosphate-buffered saline (PBS) at a concentration of 5 g/L and was stored at 4°C. The mice were injected with the NaF solution through the tail vein on the 1<sup>st</sup>, 3<sup>rd</sup>, and 5<sup>th</sup> day of the experiment. The mice received totally: 0 mg NaF/kg bw (control group), 10 mg NaF/kg bw (group 1), 50 mg NaF/kg bw (groups 2 and 3). Average body weight of the mice used in the experiment was 25 g. Each mouse received 83 µL of required NaF solution per injection. In the case of the group 1 animals, the 5 mg NaF/mL stock solution was diluted five-fold with saline, and the mice were injected with 83 µL of the resulting solution. Control mice were injected with the same volume of saline instead of NaF. The experiment was terminated after 7 days (control group and groups 1 and 2) or after 21 days (group 3). The mice were sacrificed by lethal anesthesia with sodium pentobarbitone, and the following organs were collected: liver, kidneys, spleen, and femurs.

*Microscopic examination:* For morphological studies the tissues were fixed in Carnoy's solution and stained with H-E (Hematoxylin-Eosin) and PAS (Periodic-Acid-Schiff). Fragments of the bones were decalcified<sup>17</sup> and embedded in paraffin. Microscopic slides from the various organs of the sacrificed mice were evaluated under a light microscope (Olympus, Japan) and subsequently photographed.

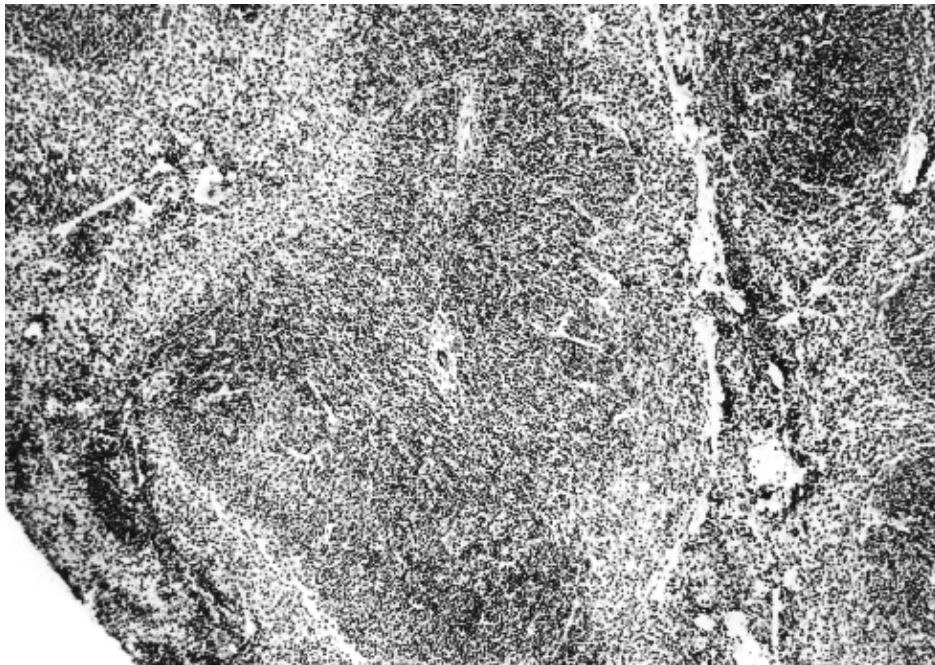
## RESULTS

The animals that were injected with higher doses of NaF (groups 2 and 3), became sick within 24 hr after the first injection. However, none of the mice died. As already stated, the mice in the control, 1<sup>st</sup>, and 2<sup>nd</sup> group were sacrificed on day 7. The animals in the 3<sup>rd</sup> group slowly recovered from the stress caused by the injection of NaF and were sacrificed on the 21<sup>st</sup> day of the experiment. Morphological findings were as follows:

*Liver:* Hepatocytes of the mice in the control group had a regular morphological structure. In the cytoplasm of the cells stained with PAS a regular distribution of glycogen was found. The most intense staining was seen in hepatocytes of the permanent repose zone around the central vein. There were no differences between liver structure of the control and experimental groups.

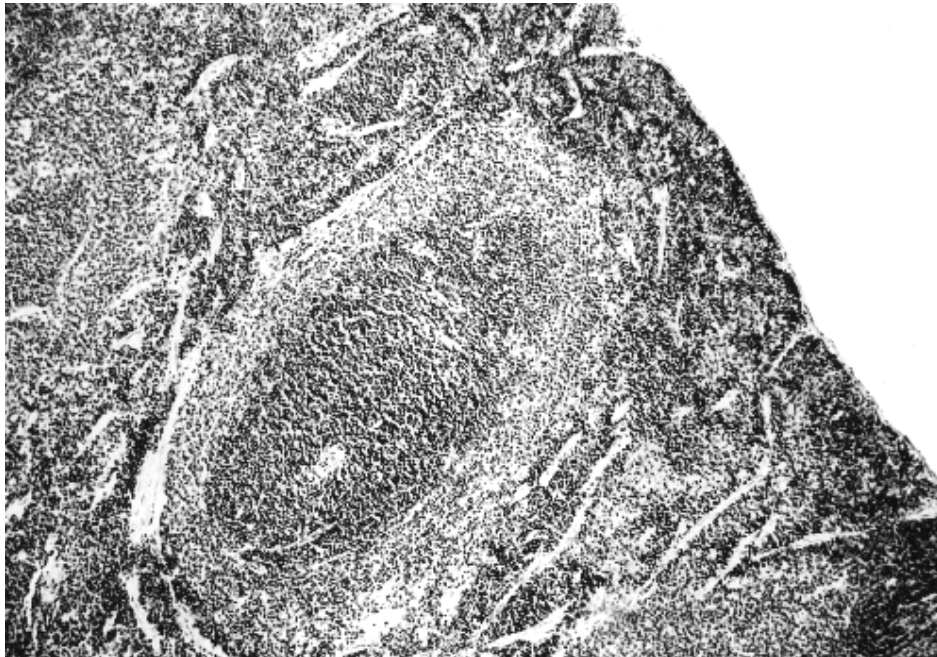
*Kidneys:* No morphological changes were detected in the kidneys, regardless of the dose and the time of the exposure.

*Spleen:* In the spleen of the control mice white pulp formed lymphoid nodules and periarterial lymphatic sheaths around central arteries (Figure 1). Red pulp occurred in the form of elongated splenic cords and splenic sinuses. Mature megacaryocytes were present.



**Figure 1.** Spleen morphology of a control mouse. H-Ex160.

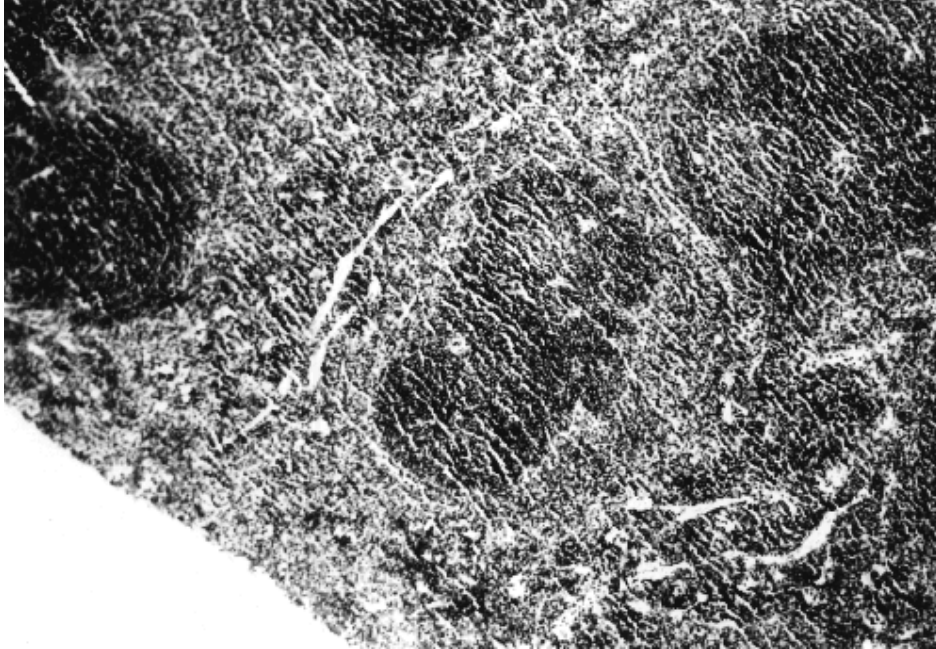
In the spleen of mice treated with NaF the amount of lymphocytes in lymphatic nodules and periarterial lymphatic sheaths of white pulp decreased. Moreover, red pulp increased and was infiltrated by lymphocytes. These changes were more intense in the spleen of mice treated with higher doses of sodium fluoride (50 mg NaF/kg bw) (Figure 2).



**Figure 2.** Spleen morphology of a mouse 7 days after the last injection of 50 mg NaF/kg bw. An increase of content of red pulp over white is visible. H-Ex160.

Figure 3 shows the spleen of animals exposed to NaF three weeks after exposure. An increase of content of red pulp over white was still visible. Wide splenic cords surrounded white pulp, and the structure of lymphatic nodules was effaced.

*Bone and bone marrow cavity:* In the femur sections, no differences in marrow cell morphology or bone structure were visible between the control and NaF exposed groups of mice.



**Figure 3.** Spleen morphology of a mouse 21 days after the last injection of 50 mg NaF/kg bw. An increase of content of red pulp over white is still visible as well as wide splenic cords surrounded white pulp and the structure of lymphatic nodules. H-Ex160.

### DISCUSSION

In these rapid-response experiments sodium fluoride caused morphological changes in the spleen, but the liver, kidneys, and bone marrow appeared to be relatively unaffected.

The spleen plays a significant role in hematopoietic system development, particularly during the fetal life. In humans, the spleen, as a site of hematopoiesis, is active mostly in fetal life.<sup>18,19</sup> In mice, on the other hand, this organ actively provides blood cell production throughout life.<sup>19</sup> Toxic damage to the spleen caused by sodium fluoride in our study may therefore have marked negative effects on hematopoiesis.

During normal development of the hematopoietic system during ontogeny the liver has an auxiliary function.<sup>18</sup> Moreover, 10 to 15% of the total pool of erythropoietin (Epo) in adults is synthesized in the liver.<sup>20,21</sup> Epo is one of the strongest growth factors regulating red blood cell production and maturation in bone marrow.<sup>22</sup> During fetal life especially the liver is a

primary source of this hormone. Recent studies in mice and rats have shown clearly that the major fraction of hepatic Epo is produced by hepatocytes.<sup>23</sup> Experimentally, in mature rodents, partial hepatectomy leads to a vigorous compensatory cellular proliferation as well as increase in hepatic Epo production, almost to the level of renal erythropoietin synthesis in control group of animals.<sup>24</sup> The same increase in hepatic Epo production is observed during the regenerative phase following toxic liver damage.<sup>25</sup>

Other hepatic factors regulating the growth and differentiation of hematopoietic progenitors are not so important as Epo. Since macrophages produce GM-CSF (Colony Stimulating Factor for Granulocytes-Macrophages), Kupffer cells (hepatic macrophages) might well also be involved in this process. However, the hepatic contribution to the regulation of these cells appears to be limited.<sup>25</sup> Moreover, the liver indirectly affects hematopoiesis by participating in the destruction of normal and abnormal blood cells. Such destruction is accomplished by the Kupffer cells, which belong to the reticuloendothelial system.<sup>26</sup> Interestingly, the most conspicuous hematological manifestation in the patients suffering from liver diseases is anemia.<sup>25</sup>

As the main organ responsible for detoxication, the liver possesses unique biochemical properties as well as huge regenerative potential. For these reasons, parenchymal tissue of the organ is characterized by relatively high resistance to sodium fluoride. According to de Camargo *et al*, rats receiving 1, 10, and 100 mg NaF/kg bw in their drinking water for 180 days did not exhibit any visible changes in the morphology of their livers.<sup>27</sup> Similar results are also reported by other authors.<sup>28</sup> Nevertheless, in the case of rabbits, a relatively high concentration of sodium fluoride (50 mg/mL in drinking water) as well as prolonged exposure (15 weeks) led to detectable necrotic changes in the organ.<sup>29</sup>

Under physiological conditions, peritubular interstitial cells of kidneys produce 85 to 90% of the total erythropoietin pool in adult humans.<sup>30</sup> The shift from hepatic to renal erythropoietin production occurs gradually at the time of birth.<sup>25</sup> Since Epo is produced mostly in the kidneys, chronic renal disease is usually associated with severe anemia.<sup>31</sup> The absence of detectable morphological changes in the kidneys of mice exposed here to NaF can be explained by both effective elimination of the microelement with urine and the relatively short period of exposure (1 week). Similar results were also obtained by others in different experimental models.<sup>27,32</sup> Nevertheless, some authors have found that longer administration of similar and higher doses of NaF led to visible morphological changes in kidneys.<sup>33,34</sup>

Bone marrow is comprised mainly of stromal and hematopoietic cells. Stroma consists of heterogenic groups of cells, mainly fibroblastic, reticular, endothelial, and adventitial, which are distinguished by their ability for dynamic renewal and regeneration of damaged fragments of the tissue.<sup>35,36</sup>

Similarly to liver and kidneys, stroma also belongs to tissues that are relatively resistant to adverse effects of sodium fluoride. However, hematopoietic cells appear to be sensitive to higher concentrations of NaF (at least 50 mg NaF/L). According to our previous findings, fluoride significantly decreases their proliferative potential.<sup>14</sup>

In conclusion, we see that fluoride is able to disturb hematopoiesis to varying degrees over a wide spectrum. Relatively high concentrations of sodium fluoride, apart from its direct influence on hematopoietic cells, can induce significant morphologic changes in the spleen.

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#### REFERENCES

- 1 Machoy, Z. Fluoride and its effect on animals and man In: *Zeszyty Naukowe PAN "Czlowiek i srodowisko"*, Ossolineum, Wroclaw; 1990. p 61-75.
- 2 Spittle B., Burgstahler A.W. Death knell for fluoridation? *Fluoride* 1998;31:59-60.
- 3 Spittle B. Unraveling the fabric of fluoridation, thread by thread. *Fluoride* 2000;32:199-200.
- 4 Spittle B. Fluoride and intelligence. *Fluoride* 2000;33:49-52.
- 5 Roholm K. Fluorine intoxication. A clinical-hygienic study. HK Lewis & Co. Ltd. London, 1937.
- 6 Ginn JT, Volker JF. Effect of cadmium and fluorine on the rat dentition. *Proc Soc Exper Biol Med* 1944;57:180-91.
- 7 McClure FJ, Kornberg A. Blood hemoglobin and hematocrit results on rats ingesting sodium fluoride. *J Pharmacol Exp Therap* 1947;89:77-80.
- 8 Uslu B. Effect of fluoride on hemoglobin and hematocrit. *Fluoride* 1981;14:38-41.
- 9 Hirao T. Blood picture of experimental fluorosis. *Fluoride* 1972;5:33-8.
- 10 Balazowa G, Macuch P, Rippel A. Effects of fluorine emissions on the living organism. *Fluoride* 1969;2:33-9.
- 11 Agate JN, Bell GH, Boddie GF, Bowler RG, Bucknell M, Cheeseman EA, et al. Industrial fluorosis. A study of the hazard to man and animals near Fort William, Scotland. London: HMSO Medical Research Council Memorandum 22; 1949.
- 12 Hoogstratten B, Leone NC, Shupe JL, Greenwood DA, Liberman J. Effect of fluorides on hematopoietic system, liver, and thyroid gland in cattle. *JAMA* 1965;192:112-8.
- 13 Hillman D, Bolenbaugh DL, Convey EM. Hypothyroidism and anemia related to fluoride in dairy cattle. *J Dairy Sci* 1979;62:416-23.
- 14 Machalinski B, Zejmo M, Stecewicz I, Machalinska A, Machoy Z, Ratajczak MZ. The influence of sodium fluoride on the clonogenicity of the human hematopoietic progenitor cells. Preliminary report. *Fluoride* 2000;33:168-17.
- 15 Machalinska A, Machoy-Mokrzynska A, Marlicz W, Stecewicz I, Machalinski B. NaF-induced apoptosis in human bone marrow and cord blood CD34 positive cells. *Fluoride* 2001;34:258-63.
- 16 Machalinska A, Nowak J, Jarema A, Wiszniewska B, Machalinski B. In vivo effects of NaF on bone marrow transplantation in lethally irradiated mice. *Fluoride* 2002;35:81-9.

- 17 Pearse AGE. Histochemistry. Theoretical and applied. In: J. & A. Churchill Ltd; 1961. p 776.
- 18 Sieff CA, Williams DA. Hematopoiesis. In: Handin RI, Stossel TP, Lux SE, editors. Blood: Principles and Practice of Haematology. Philadelphia: JB Lippicott Company; 1995. p. 171-224.
- 19 Sty JR, Conway JJ. The spleen: development and functional evaluation. *Semin Nucl Med* 1985;15:276-98.
- 20 Shoemaker CB, Mitscock LD. Murine erythropoietin gene: cloning, expression, and human gene homology. *Mol Cell Biol* 1986;6:849-58.
- 21 Koury ST, Bondurant MC. The molecular mechanism of erythropoietin action. *Eur J Biochem* 1992;210:649-56.
- 22 Graber SE, Krantz SB. Erythropoietin: biology and clinical use. *Hematol Oncol Clin North Am* 1989;3:369-400.
- 23 Koury ST, Bondurant MC, Koury MJ, Semenza GL. Localization of cells producing erythropoietin in murine liver by in situ hybridization *Blood* 1991;77:2497-2503.
- 24 Anagnostou A, Schade S, Barone J, Fried W. Effects of partial hepatectomy on extrarenal erythropoietin production in rats. *Blood* 1977;50:457-462.
- 25 Erslev AI. The hemopoietic functions of the liver. In: Arias IM, Boyer JL, Fausto N, Jacoby WB, Schachter D, Shafritz DA, editors. The liver: biology and pathobiology. 3rd ed. New York: Raven Press; 1994. p. 1227-34.
- 26 Gale RP, Sparkes RS, Golde DW. Bone Marrow Origin of Hepatic Macrophages (Kupffer Cells) in Humans. *Science* 1978;201:937-938.
- 27 de Camargo AM, Merzel J. Histological and histochemical appearance of liver and kidneys of rats after long term treatment with different concentrations of sodium fluoride in drinking water. *Acta Anat* 1980;138:288-94.
- 28 Olgivie AL. Histological findings in the kidney, liver, pancreas, adrenal and thyroid gland of the rat following administration of sodium fluoride. *J Dent Res* 1953;32:386-97.
- 29 Shashi A, Thapar SP. Histopathology of fluoride-induced hepatotoxicity in rabbits. *Fluoride* 2001;34:34-42.
- 30 Lacombe C. et al. Peritubular are the site of erythropoietin synthesis in the murine hypoxic kidney. *J Clin Invest* 1988;81:620-5.
- 31 Chandra M, Clemons GK, McVicar MI. Relation of serum erythropoietin levels to renal excretory function: evidence for lowered set point for erythropoietin production in chronic renal failure. *J Pediatr* 1988;113:1015-21.
- 32 Manocha SL, Warner H, Olkowski Z. Cytochemical response of kidney, liver and nervous system of fluoride ions in drinking water. *Histochem J* 1975;7:343-55.
- 33 Kour K, Singh J. Histological findings in kidneys of mice following sodium fluoride administration. *Fluoride* 1980;13:163-7.
- 34 Shashi A, Singh JP, Thapar SP. Toxic effects of fluoride on rabbit kidney. *Fluoride* 2002;35:38-50.
- 35 Dorshkind K. Regulation of hemopoiesis by bone marrow stromal cells and their products. *Annu Rev Immunol* 1990;8:111-37.
- 36 Muller-Sieburg ChE, Deryugina E. The stromal cells guide to the stem cell universe. *Stem Cells* 1995;13:477-86.