

# CHANGES IN CALCIUM AND PROTEIN METABOLISM IN THE BONES AND TEETH IN HYPERCAPNIA

A. I. Volozhin and T. I. Lemetskaya

UDC 616:61+616.314]-008.9-  
02:612.232.4

Under the influence of gradually increasing hypercapnia (3-5% CO<sub>2</sub>) in rats the rate of loss of Ca<sup>45</sup> from the bones and teeth is increased. Inhibition of synthesis in the bone tissue is accompanied by marked dilatation and congestion of the vessels in the bone tissue itself, the bone marrow, and the dental pulp.

An increase in the carbon dioxide concentration in the inspired air leads to a decrease in the rate of assimilation of calcium in bone tissue, delayed growth of the skeleton, and the deposition of calcium salts in the kidney tissue [4]. The mechanism of these changes has not been studied.

The object of this investigation was to study the effect of periodic hypercapnia on the calcium and protein metabolism in the bones and dental tissues.

## EXPERIMENTAL METHOD

Experiments were carried out on 82 noninbred male albino rats weighing initially 180-195 g. The animals were divided into two groups; experimental and control. Hypercapnia was produced in the experimental rats for 6 h daily by adding CO<sub>2</sub> to the atmosphere in a pressure chamber in sufficient quantity to give a concentration of 3% on the first three days, 4% on the next three days, and 5% on the seventh and subsequent days. The calcium and protein metabolism was studied on the twentieth and thirtieth days of exposure. For this purpose the animals of both groups were divided into two subgroups: in subgroup 1 the excretion of Ca<sup>45</sup> from the bones and teeth was studied and a histological investigation of these structures carried out; in subgroup 2 the incorporation of Ca<sup>45</sup> and glycine-2-C<sup>14</sup> was studied by the usual method [3]. The rats of subgroup 1 received Ca<sup>45</sup> for the ten days before exposure, and on the day the experiment began six animals were killed, the molar and incisor teeth and the mandibles, femora, and tibiae were excised and analyzed for their content of the isotope (background). The animals of subgroup 2 received Ca<sup>45</sup> by subcutaneous injection 24 and 48 h before sacrifice and glycine-2-C<sup>14</sup> 24 h before sacrifice. The calcium [2], inorganic phosphorus [1], and total protein (by Lowry's method) were determined in the blood plasma after decapitation.

Bone tissue for histological investigation was fixed in 12% neutral formalin, decalcified in 7% nitric acid, and the sections were stained with hematoxylin-eosin and by Van Gieson's method.

## EXPERIMENTAL RESULTS

Hypercapnia had no effect on the concentration of calcium, inorganic phosphorus, and total protein in the rats' blood. The weight of the animals of the experimental groups was 8.3% below that of the control ( $P < 0.05$ ). The weight of the teeth and bones of the rats was unchanged after hypercapnia, but the weight index was increased by 3-9% ( $P < 0.05$ ).

---

Department of Pathological Physiology, Moscow Medical Stomatological Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 75, No. 5, pp. 42-43, May, 1973. Original article submitted November 4, 1971.

©1973 Consultants Bureau a division of Plenum Publishing Corporation, 227 West 17th Street, New York, N. Y. 10011. All rights reserved. This article cannot be reproduced for any purpose whatsoever without permission of the publisher. A copy of this article is available from the publisher for \$15.00.

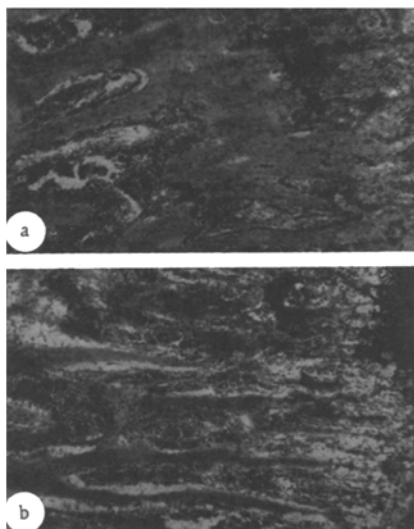


Fig. 1. Photomicrograph of the femur of a rat: 1) control; b) hypercapnia 30 days: decrease in number and thickness of bone trabeculae, dilatation of blood vessels. Hematoxylin-eosin, 56 $\times$ .

Radiometric investigations showed that the  $\text{Ca}^{45}$  concentration in the molar and incisor teeth, and the mandibles, femora, and tibiae of the rats before the beginning of the experiment (background) was  $0.109 \pm 0.0069$ ,  $1.083 \pm 0.103$ ,  $0.959 \pm 0.030$ ,  $1.966 \pm 0.097$ , and  $0.980 \pm 0.091\%$  of the injected dose of isotope, respectively. The concentration of  $\text{Ca}^{45}$  in the molars of the control rats of subgroup 1 was regularly increased on the twentieth and thirtieth days of observation (135 and 151% of the background values;  $P < 0.01$ ), while after hypercapnia the change was smaller (106%,  $P > 0.1$ ; 119%,  $P > 0.05$ ). The incorporation of  $\text{Ca}^{45}$  into the molars of the rats of the control and experimental groups was the same.

The concentration of  $\text{Ca}^{45}$  in the incisors of the control and experimental animals was increased on the twentieth day (134 and 144% of the background values;  $P < 0.01$ ), while on the thirtieth day it remained high in the control but was considerably reduced in the animals exposed to hypercapnia (84.8% of the background). The incorporation of  $\text{Ca}^{45}$  into the incisors of the rats was increased under the influence of hypercapnia, especially on the thirtieth day (113 and 159% of the control 24 and 48 h respectively after administration of the isotope). The calcium metabolism in the mandibles was not significantly changed. In the femora an increase in the rate of  $\text{Ca}^{45}$  resorption was observed under the influence of hypercapnia (by 6% over the control) and the incorporation of the isotope was increased on the twentieth day of the experiment (119% of the control value after 48 h;  $P < 0.05$ ). Similar results were obtained by investigation of the tibiae.

Incorporation of glycine-2- $\text{C}^{14}$  into total acid-insoluble proteins of the experimental animals was reduced on the twentieth day (76% of the control;  $P \geq 0.05$ ), and into the proteins of the femora it was reduced on the twentieth and thirtieth days of the experiment (88 and 81% of the control;  $P < 0.05$ ). Incorporation of  $\text{C}^{14}$  into proteins of the tibiae was reduced on the thirtieth day but not by a statistically significant degree. No change was observed in the protein metabolism in the incisors of the rats exposed to hypercapnia, while in the mandibles the incorporation of  $\text{C}^{14}$  was increased on the thirtieth day of exposure (118% of the control;  $P < 0.05$ ).

Histologically, inhibition of the remodeling of the bone tissue was observed on the twentieth day of hypercapnia; the number of osteoblasts was reduced, and sometimes none whatever could be seen. Inclusions of cartilage were seen more often than normally in the bone trabeculae and the blood vessels were dilated and congested. No significant changes were present in the epiphyseal cartilage. On the thirtieth day of hypercapnia the vascular reaction in the femora (Fig. 1) was more marked than on the twentieth day. Groups of osteoclasts could be seen in zones of resorption of the bone tissues. Some increase in the intensity of osteoclastic resorption of bone tissue against the background of marked hyperemia was noticed in the mandibles of the experimental animals. The blood vessels of the pulp of the molar and incisor teeth also were dilated and congested.

These results show that under the influence of gradually increasing hypercapnia (3-5%  $\text{CO}_2$ ) the rate of resorption of  $\text{Ca}^{45}$  from the bones (except the mandibles) and teeth of rats is increased. Inhibition of synthesis in the bone tissue is accompanied by dilatation and congestion of the blood vessels.

#### LITERATURE CITED

1. A. I. Volozhin, *Kosmich. Biol. i Med.*, No. 2, 17 (1971).
2. A. S. Kantorovich and L. I. Belinskaya, *Lab. Delo*, No. 5, 282 (1965).
3. A. A. Prokhonchukov and Lie Ting-hsin, *Med. Radiol.*, No. 7, 70 (1961).
4. K. E. Schaefer, *Ann. New York Acad. Sci.*, 109, 772 (1963).