Effects of acute intoxication with uranyl nitrate on bone formation[1]

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Summary. The alteration of bone formation by an acute intoxication with uranyl nitrate is demonstrated by histologic and histometric methods. When compared with the controls, intoxicated animals showed a markedly lower density in healing sockets, while bone formation was reduced in healing sockets as well as in metaphyseal bone.

The incorporation of uranium compounds by bone tissue has been demonstrated by different methods; biochemical[2-4], autoradiographic[5-7], neutron activation analysis[8] and X-ray microanalysis[9]. A large proportion of the uranium injected is deposited in bone tissue, in which uranium is found in significant amounts after long periods of time[3], and it is considered the critical organ in chronic exposures[4]. Autoradiographic studies have demonstrated the primary deposition of uranium on endostal, periostal and haversian surfaces[7], particularly in calcifying zones. All this information clearly indicates a relationship between uranium deposits and bone formation. Nevertheless, there is no reference to the changes produced in bone tissue by uranium intoxication. Here we present a histologic and histometric study concerning the effect of acute intoxication with uranyl nitrate on formation of bone tissues.

Material and methods. 22 male Wistar rats weighing 90 g were employed. All animals had the 3 left mandibular molars extracted under Nembutal anesthesia (0.0025 g/kg b.wt). 11 of these animals were injected i.p. with 2 mg/kg b.wt of uranyl nitrate immediately after the extractions. The remaining non-intoxicated animals were used as controls. All animals were killed on the 14th day by ether inhalation.

Tibiae and jaws were fixed in 10% neutral formalin, decalcified in EDTA and processed to be embedded in paraffin. Oriented sections were stained with hematoxylin-eosin. Histometric measurements were made on projections of these sections using an image analyzing system (Kontron Messgeräte MOP/AM 03 Carl Zeiss). Bone formation zones were observed microscopically and drawn in the corresponding zones of the tracings.

The measurements were performed on the central area of metaphyseal bone (fig. 1) and on the apical third of the first molar mesial root in the bucco-lingual middle area. The histometric analysis was based on standard stereological methods[10-12].

The parameters studied in tibiae were: a) percentage of bone formation surface of the total trabecular bone, considering bone formation surfaces as those covered by osteoid tissue and cuboidal osteoblasts; b) volume density of trabecular bone (Vv), considered as the ratio between trabecular area and total area; c) width of trabeculae taken on the line GH traced parallel to segment EF in the middle of zone I. In the sockets the parameters analysed were: a) percentage of bone formation surface and b) volume density of trabecular bone.

Results. The histologic sections of tibiae showed an altered architecture of the metaphyseal zone in injected animals. The most striking finding was a marked shortening of the metaphyseal bone. Their trabeculae were shorter and wider (sealing trabeculae) than in the controls, while osteoblastic areas were less frequent.

The histometric analysis demonstrated that the percentage of bone formation areas in the central zone of metaphyseal bone was significantly lower in experimental than in control animals, particularly in sector III (table 1, fig. 1).

The mean values of the volume density of trabecular bone in zone CDEF were 0.21 ± 0.03 for controls and 0.24 ± 0.02 for experimental animals ($p < 0.005$) (table 2). Nevertheless, in sector III of that zone, Vv was significantly lower in experimental animals than in controls (fig. 1).

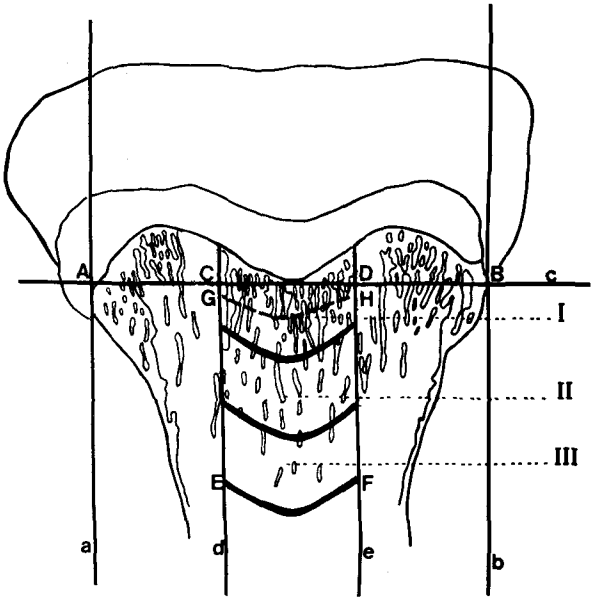

Figure 1. The area CDFE considered for the measurements was traced on the projections. Two lines a and b parallel to the diaphysis and tangents to the point of union between the cortical-bone and the epiphyseal cartilage were drawn. Line c was drawn perpendicular to these lines and tangent to the lower edge of the epiphyseal cartilage. Segment AB was thus determined and divided in thirds: AC, CD and DB. The lines d and e were drawn parallel to the lines a and b from the points C and D. A line parallel to the lower edge of the cartilage was drawn 24 cm from it, defining points E and F where it intersects lines d and e. The 24 cm are equivalent of 4000 µm, which correspond to the mean value of the distance between the lower edge of the cartilage and the lower end of the bone trabeculae obtained from controls. The central sector of subchondral bone is thus limited by the points C,D,F and E. This area was divided into three zones: I, II and III by dividing the altitude DF into thirds and drawing lines parallel to the lower edge of the cartilage.

Table 1. Histometric analysis in subchondral zone. Percentage of bone formation surfaces

	Control animals	Experimental animals	p
Sector I	95.43 ± 1.11	97.67 ± 0.75	< 0.01
Sector II	3.53 ± 0.90	2.20 ± 0.52	< 0.025
Sector III	1.01 ± 0.67	0.12 ± 0.24	< 0.025
Mean value	24.63 ± 4.33	19.99 ± 2.08	< 0.001

All values ± SD.

Table 2. Histometric analysis in subchondral zone. Volume density of trabecular bone

	Control animals	Experimental animals	p
Sector I	0.48 ± 0.05	0.56 ± 0.03	< 0.05
Sector II	0.13 ± 0.04	0.16 ± 0.04	< 0.05
Sector III	0.04 ± 0.01	0.008 ± 0.01	< 0.001
Mean value	0.21 ± 0.03	0.24 ± 0.02	< 0.005

All values ± SD.

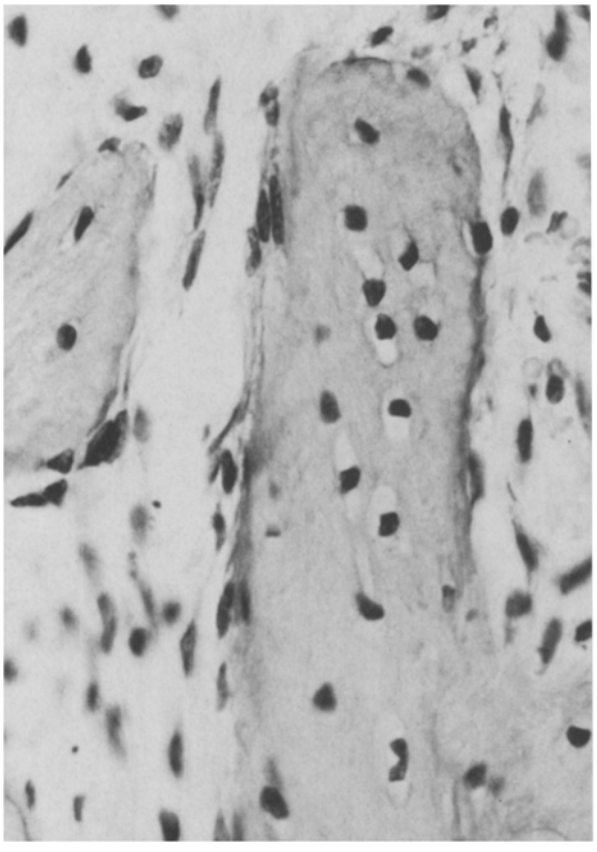

Figure 2. Trabecula corresponding to the healing socket of an intoxicated animal. No forming activity is showed on its surface. HE ×700.

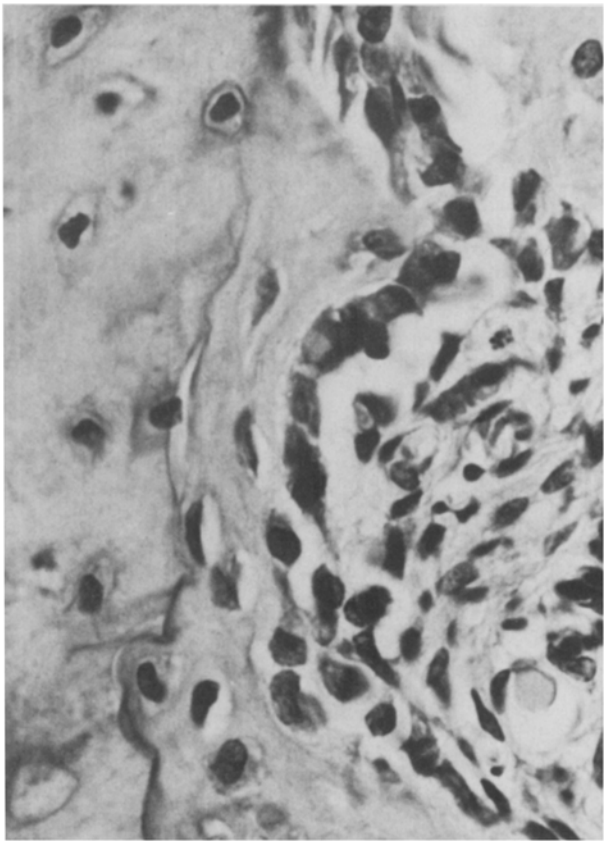

Figure 3. Trabecula corresponding to the healing socket of a control animal. It shows a row of cuboidal osteoblasts covering the osteoid tissue. HE, ×700.

The trabecular width measured in sector I (fig. 1) was greater in injected animals (4.11 mm ± 0.41 SD) than in controls (2.66 mm ± 0.17 SD). This fact explains the greater values of Vv for sectors I and II in zone CDEF (fig. 1).

The sockets of intoxicated animals were almost completely occupied by proliferating connective tissue. A few woven trabeculae with scanty bone formation surfaces were observed occupying the apical alveolar third. Sockets of non-injected animals were almost totally occupied by woven bone with high osteoblastic activity (figs 2 and 3). The histometric determinations in the apical third of the extraction wound healing socket of the injected animals demonstrated that the percentage of bone formation surfaces as well as bone density were remarkably lower than in the controls (table 3).

Discussion. Our results indicate that acute intoxication with uranyl nitrate provokes important alterations in bone formation. This effect was observed on endochondral ossification as well as in a model of bone healing of edentate alveoli. These results are supported by histologic and histometric analysis. The use of histometric methods allows the quantitative evaluation of bone alterations, as has been demonstrated elsewhere[10-12]. With these methods it was determined that bone formation surfaces were significantly reduced in intoxicated compared with control animals, both in the metaphyseal bone and in healing sockets.

Table 3. Histometric analysis in healing sockets

	Control animals	Experimental animals	p
Percentage of bone formation surfaces	19.55 ± 4.00	4.85 ± 2.60	< 0.001
Volume density of bone in the apical third	0.40 ± 0.06	0.26 ± 0.04	< 0.001

All values ± SD.

The metaphyses of intoxicated animals showed signs of depressed ossification as revealed by the lower percentage of bone formation areas when compared to the controls. It was significantly lower particularly in sector III ($p < 0.001$). Volume density of metaphyseal bone was significantly lower in sector III, due to the diminished length of its trabeculae, which causes a virtual absence of bone. Sector III may be considered representative for studying the effect of uranium on growth of epiphyseal bone because it may be assumed that their trabeculae have been formed during the first 3–4 days after the injection. These results are in accordance with the findings in the alveolar sockets and reflect diminished osteoblastic bone formation. The formation of woven trabeculae, which normally occupy the alveolus 14 days after tooth extraction[13], was virtually absent in intoxicated animals, thus resulting in a lower volume bone density.

In both cases it may be assumed that the differentiation of osteoblasts or their precursors is depressed by the toxic agent. Concomitantly, the remaining osteoblasts may form the scanty woven trabeculae found in the alveolar fundus and the sealing trabeculae which appeared in the metaphyseal bone. The presence of sealing trabeculae in sector I indicates that bone formation has stopped and explains the increment in the value of bone density found in this sector. On the other hand the decrease of bone resorption may have contributed to these results.

Under similar experimental conditions cell damage has been reported in kidney and skin[14,15]. Based on these data and on the demonstrated relationship between bone formation and uranium deposits[3,5-7] osteoblastic or preosteoblastic damage cannot be excluded.

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The effect of ether, pentobarbitone sodium and fentanyl on blood gases, acid-base balance and hematological parameters in the rat

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Summary. Blood gases, acid-base balance and hematological parameters (RBC, PCV and Hb) were measured in adult rats of both sexes. The use of ether and fentanyl had a very little effect on the blood gases and acid-base balance. The induction of pentobarbitone anesthesia, however, was followed by a significant increase in PCO_2 and TCO_2, while the pH value decreased.

Many investigations have been performed in an attempt to provide normal values for the blood gases and the acid-base balance of the rat[2-8], but there have been surprisingly few carefully-controlled studies comparing the effects of the anesthetics and mode of blood sampling on these parameters. It has been reported that the blood values of anesthetized rats may not accurately reflect the acid-base status in conscious ones[9]. Therefore we performed experiments to compare the effects of anesthesia and analgesia on hematological parameters, blood gases and acid-base balance with the influence of manual restraint.

Materials and methods. Studies were made using adult rats of both sexes (Tif: RAIf, Ciba-Geigy) weighing between 220 and 300 g, which were kept under S.P.F. conditions with access to food and water ad libitum. The animals were fasted overnight, from about 16.00 h until the experiment was started around 08.00 h the next morning. Either manual restraint or anesthesia was employed while postorbital puncture (retroorbital venous plexus) was carried out to remove blood from each animal; it was drawn anaerobically before the anesthetic (ether or pentobarbitone sodium) or analgesic agent (fentanyl) was administered (pre-treatment values), and a 2nd sample taken as the rat succumbed to anesthesia or analgesia (treatment values), i.e. when drowsiness became apparent. The time point at which the 2nd blood sample was taken was identical for all 3 drugs. To prevent hypothermia the rats were placed in an insulated container. Rectal temperature was taken in some animals randomly selected and was found to be within normal limits (normothermia) for this strain of rat.

Ether anesthesia was induced by placing the rat ($n=30$) in a covered glass jar containing a pad of cotton wool soaked in diethylether (Aether ad narcosin, Siegfried, Switzerland). Pentobarbitone sodium (Nembutal, Abbott Laboratories, USA) was administered by i.p. injection using a dose of 5 mg/100 g b.wt ($n=20$).

Analgesia ($n=20$) was produced by i.p. injection of 0.1 ml fentanyl solution/100 g b.wt (Fentanyl Janssen, Belgium). Each ml of the solution containing 0.05 mg of the analgesic and sedative fentanyl.

Because of the limited blood sampling that can be done in the rat without the risk of interfering with the hematological parameters, a separate study was made on 15 control animals to check its influence on the values being measured.

Blood gases and acid-base balance were analyzed immediately after sampling by an electrode system (micromethod, Blood Gas Analyzer, Corning, USA). Two buffer solutions and 2 gas mixtures were used for calibration. All hematological measurements (RBC, PCV and Hb) were made with the Coulter Counter S-Plus (Coulter Electronics, Inc., Florida, USA) which was calibrated in the standard manner.

The t-test[10] for differences between paired observations was used to analyse these data, and significance was accepted at the 1% level.

Results. The results are expressed as means and SD. Effects of anesthesia and analgesia: Table 1 shows the values obtained for blood gases, acid-base balance and hematological parameters measured immediately before and during anesthesia or analgesia. The use of ether and fentanyl had very little effect on the blood gases and acid-base balance; except for the increase ($p < 0.01$) in PO_2 caused by ether, there were no significant differences between conscious (before anesthesia) and anesthetized animals. In the present experiment, pentobarbitone narcosis was followed by an increase in PCO_2 ($p < 0.01$), TCO_2 ($p < 0.01$) and bicarbonate level ($[HCO_3^-]$) in the blood, while the