Journal of Oral Pathology 1985: 14, 565–572

Alveolar wound healing alteration under uranyl nitrate intoxication

M. B. Guglielmotti, A. M. Ubios and R. L. Cabrini

Department of Radiobiology, National Atomic Energy Commission; CONICET and Department of Pathology, Faculty of Dentistry, University of Buenos Aires, Argentina.

The deposit of uranium compounds in calcifying zones has been demonstrated in bone. Nevertheless, no studies on the effect of uranium on osteogenesis have been performed. A histologic and histometric study of the effect of a single intraperitoneal injection of 2 mg/kg of body weight of uranyl nitrate on bone formation is presented. It was performed on rats' healing sockets, 14 days after tooth extraction. The alveolar bone volume (15×10^5 μm^2 vs. 34×10^5 μm^2), total bone formation areas (4.85% vs. 19.55%), and volume density of bone in the alveolar apical third (0.26 vs. 0.40) were significantly lower in intoxicated animals than in the controls. These results indicate the inhibitory effect of uranyl nitrate on bone formation.

Accepted for publication October 15, 1984

Alterations of different tissues of the body, particularly of skin and kidney, induced by uranium compounds have been described in the literature (1–6). In bone, the distribution and retention of uranium compounds were studied by different methods: biochemical (7–9), and autoradiographic (10–12), by neutron activation analysis (13), and X-ray microanalysis (6). Autoradiographic studies have demonstrated primary deposition of uranium on endosteal, periosteal and Haversian surfaces (12), particularly in calcifying zones (10). Bone is the only tissue in which uranium is found in significant amounts after long periods of time (8), and is considered the critical organ in chronic exposures (9).

All this information clearly indicates a relationship between deposits and bone formation. Nevertheless, no reports on the possible effects on osteogenesis induced by uranium compounds are available in the literature. Preliminary data on the depression of osteoblastic activity in jaw bone and endochondral ossification of tibia produced by an acute intoxication with uranyl nitrate have recently been reported by us (14).

The aim of this experiment was to study radiographically, histologically and histometrically, the effect of the acute intoxication with uranyl nitrate in a forming bone. For that reason post-extraction alveolar wound healing was used as a model of active osteogenesis.

Material and methods

Twenty-two male Wistar rats weighing 90 g were anesthetized with an intraperitoneal injection of sodium pentobarbital (0.025 g/kg of body weight). In order to obtain a representative tissue with active osteogenesis free of interferences, the 3 left mandibular molars were extracted.

The extractions were performed with the aid of two enamel hatchets; one of them was used as a dental elevator, the other was sharpened for use as a syndesmotome. In all cases the complete extraction of the 3 molars was achieved. No bone adhered to the extracted teeth. This fact was corroborated by observation under a stereoscopic microscope. Neither special diet nor antibiotics were administered.

After the extractions the animals were separated into 2 groups. One group of 11 control animals received no further treatment. The second group of 11 experimental animals immediately post-extraction were injected intraperitoneally with 2 mg/kg body weight of uranyl nitrate (^{238}U) dissolved in trismaleate buffer pH 7.2 (0.02% w/v).

All the animals were killed 14 days after the tooth extractions by ether inhalation. The jaws were fixed in 10% v/v formalin solution radiographed using mammagraphic film and decalcified in EDTA 10% w/v pH 7. The left and right jaws were then placed side by side and a buccolingual section, parallel to the mesial surface of the right first molar, was performed. The left jaws were embedded in paraffin, sectioned and stained with hematoxylin and eosin. The first molar mesial socket was chosen as it is one of the biggest of the three mandibular molars.

Histometric measurements based on standard stereological methods (15–20) were performed using an image analysing system (Kontron MOP AM 03) on tracings obtained from projections of the sections. Bone formation and bone resorption zones observed microscopically were transferred to the corresponding zones on the tracings.

The following histometric determinations were carried out:

I) *Total alveolar volume:* considered as the bone tissue and its marrow spaces situated above a line *a* drawn tangential to the upper cortical portion of the mandibular canal (point A) and perpendicular to the external surface of the buccal plate (Fig. 1).

II) *Volume density:* considered as the ratio between the trabecular area and the bone area measured in the rectangle ACFI outlined on the apical third of the socket (Fig. 2). This was determined as follows: Point A: as was described in I.

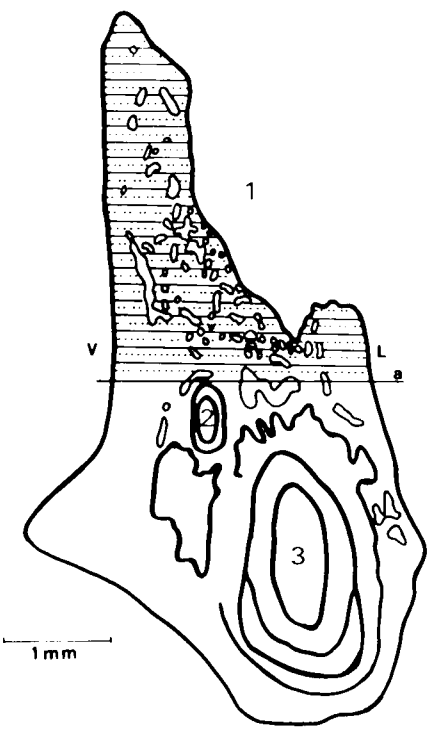

Fig. 1. Dotted zone represents the area measured for the total alveolar volume above line *a*. V: vestibular plate; L: lingual plate; 1: alveolus; 2: mandibular canal; 3: growing incisor.

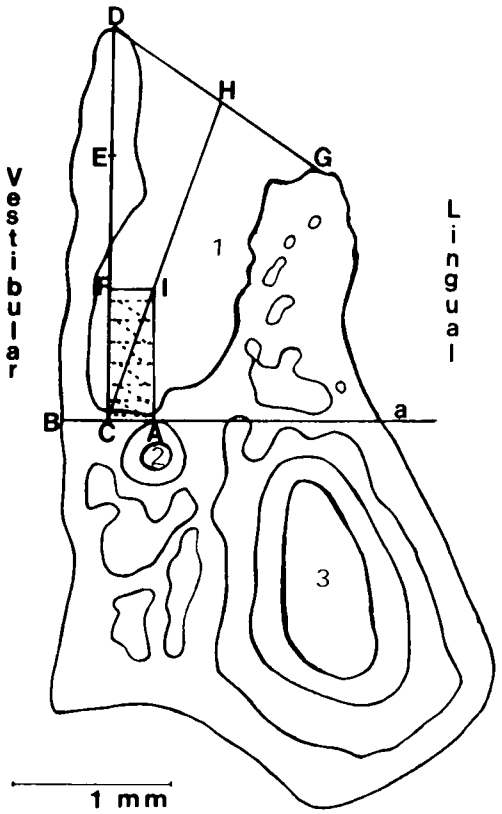

Fig. 2. The drawing represents the alveolus (1) immediately after the extraction. Points ACFI determine the rectangle used for the measurements in the apical third. (2): mandibular canal; (3): growing incisor.

Point C: on the line *a* equidistant from A and B. Intersection of line *a* and the external buccal plate gave rise to point B.

Point F: the segment obtained by joining C with the top point of the vestibular crest (D) was divided in thirds. This determined points E and F.

Point I: the segment DG was drawn joining the top point of the lingual crest (G) with point D. Point H was determined on the middle of this segment, thus, giving rise to segment HC which coincides with the middle of the socket. A line perpendicular to segment CD was drawn from F to the intersection with segment HC giving rise to point I and segment IA.

III) *Percentage of bone formation and bone resorption areas:* both were measured on the bone surface of marrow spaces and on the bone surface of the alveolar ridge. Those areas covered by an osteoid band with cuboidal osteoblasts were considered as bone formation areas, while erosive zones were considered as bone resorption areas and the remaining bone was considered as bone at rest. Bone formation and bone resorption areas were determined separately in the total bone surface situated above the line *a* (Fig. 1) and in the rectangle ACFI (Fig. 2).

The absolute values obtained from the areas of resorption, formation and rest were then expressed as a percentage of the total bone perimeter of marrow spaces and alveolar ridge.

The histometric values obtained were analyzed statistically using the nonparametric Mann-Whitney U test (21), values of $P < 0.004$ were accepted as being statistically significant. Only one section of each block was used. It has been demonstrated (17) that data thus obtained, adequately represent the parameters measured and are suitable for statistical purposes. We have recently reported that the use of this methodology for the study of alveolar wound healing affords highly uniform values (20).

Results

The roentgenographic appearance was of a homogeneous radioopaque area occupying the edentate alveolar ridge of control animals 14 days after the molar extractions, whereas in injected animals the alveoli were clearly distinguishable by their radioluscent images (Fig. 3).

This histological study showed a great dif-

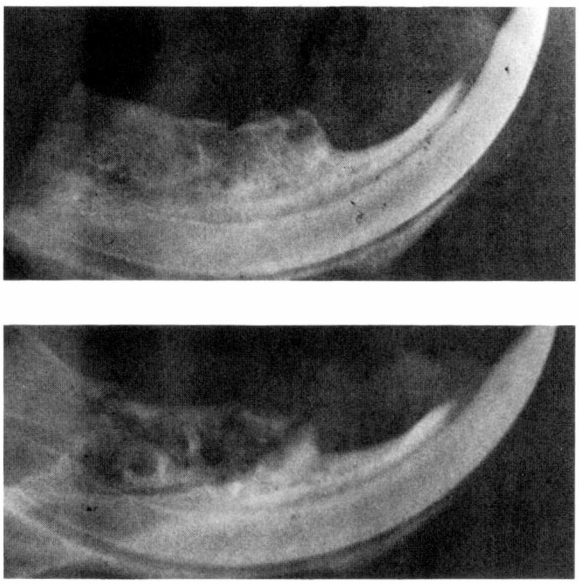

Fig. 3. Radiographic appearance 14 days after the extraction. Upper: control; lower: experimental.

ference in the alveolar structure of experimental animals when compared to their controls, as the tracings in Fig. 4 show. The histologic sections corresponding to control animals showed a socket almost totally filled by reticular bone whose trabecular surfaces showed an intense bone activity. Bone formation areas were observed both on the bone surface of marrow spaces and on the bone surface of the alveolar ridge. Over the woven trabeculae a mature connective tissue was observed while the overlying mucous membrane was completely healed.

In injected animals the histological study showed that the alveolus was almost totally occupied by granulation tissue. Only on the apical fundus of the alveolus newly formed woven trabeculae were seen. In 5 of the 11 cases, cartilage tissue was observed on the external surface of vestibular crests (Fig. 5).

In the upper zone of the lingual crest of both control and experimental animals, few bone resorption areas with typical osteoclasts were presented while no bone resorption areas were seen in the apical third.

Histometrical measurements performed in the total alveolous as well as those obtained in the apical third showed a clear inhibition of bone formation in intoxicated animals (Table 1).

Total bone volume, volume density and bone formation surfaces were significantly lower in injected than in control animals. The differences between all these measurements were statistically significant in every case.

No differences in bone resorption areas were established.

Discussion

Alveolar wound healing provides a suitable model for the study of bone formation and can be considered a sensitive indicator of bone damage under different experimental conditions. Complete extraction of rat molars

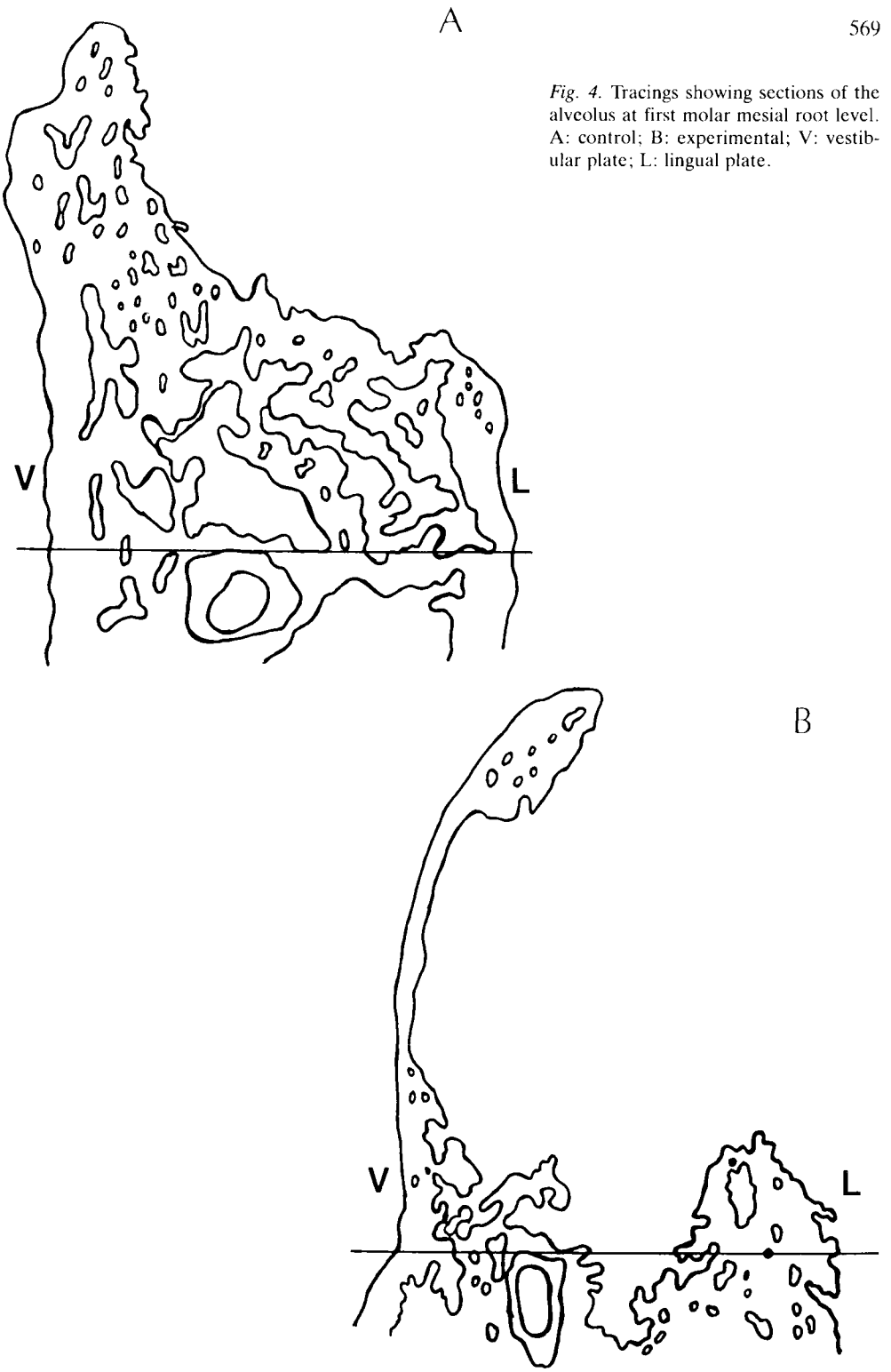

Fig. 4. Tracings showing sections of the alveolus at first molar mesial root level. A: control; B: experimental; V: vestibular plate; L: lingual plate.

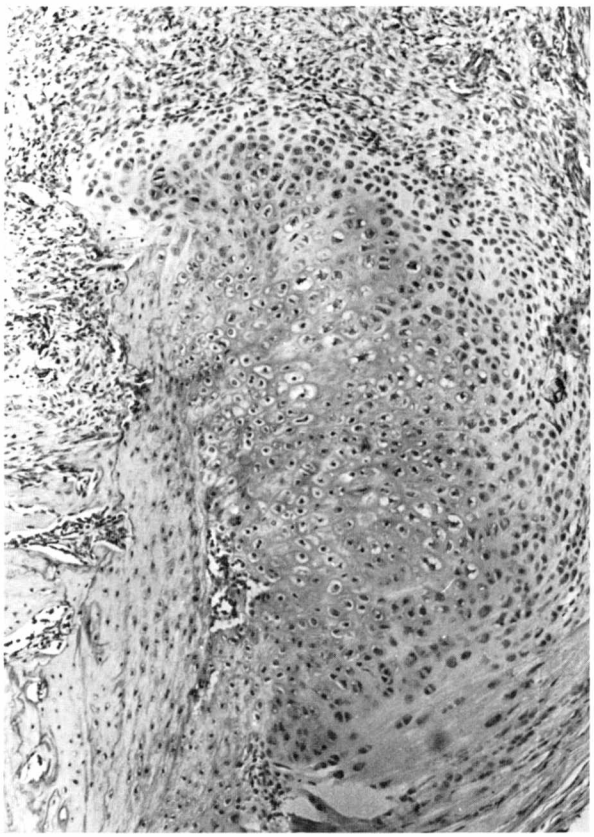

Fig. 5. Cartilage was observed in some cases of injected animals on the external surface of the vestibular crest.

Table 1. Histometric study in 14th-day healing sockets.

	Non-intoxicated animals	Animals intoxicated with uranyl nitrate	p U test
Total bone volume	34×10^5 μm^2	15×10^5 μm^2	0.004*
Volume density of bone (Apical third)	0.40	0.30	0.004*
Percentage of bone formation (Total alveolus)	19.00	5.00	0.004*
Percentage of bone resorption (Total alveolus)	1.00	1.00	0.4605
Percentage of bone formation (Apical third)	33.00	10.00	0.004*
Percentage of bone resorption (Apical third)	–	–	–

*statistically significant.

was reported to be quite difficult (22–24), but the surgical technique used in this work avoided such a problem. It offers the possibility of studying the process of the alveolar wound healing after tooth extraction without the interference of radicular rests.

In a previous histologic and histometric study the greatest proportion of bone formation in alveolar wound healings in rats was proved to appear 14 days after tooth extraction (20).

Data obtained in this experiment clearly indicated that uranyl nitrate alters the healing of sockets after tooth extraction by inhibition of bone formation. The use of standard histometric methods (18) repeatedly proved in alveolar bone (15,16,19,20) revealed this effect in every parameter considered.

The curious cartilage formation found in the vestibular crest in 5 of 11 cases could be due to a chondroblastic differentiation of periosteal osteoblasts. It could be explained as a delay in bone formation associated with the mechanical action of the masticatory activity or by non-specific toxic action of the uranyl nitrate.

Most of the uranium isotopes are considered to be a chemical rather than a radiological hazard (9,11). There are extensive data on the toxicity of uranium compounds in different tissues (1–4,9,11). Because the radioactivity of the uranyl nitrate can be considered negligible, the alterations in bone formation found here could be attributed to the chemical toxicity of the heavy metal.

Twenty-five percent of the uranium administered systemically is deposited in the skeleton (23). Its distribution is quite similar to that of calcium (11) while it binds as "hot spots" to newly formed bone (11,12). In chronic exposures to uranium, bone retains the greatest amount and replaces kidney as the critical organ (9). Taking into account this data, and the cellular alterations found in kidney caused by uranyl nitrate (5) and in skin caused by soluble and insoluble uranium salts (6) it could be inferred that the acute intoxication with uranyl nitrate could cause preosteoblastic and osteoblastic damage. This could explain the inhibition of bone formation found here.

Acknowledgements

This work was supported in part by Grant 8461 d/2 of the Consejo Nacional de Investigaciones Científicas y Técnicas Argentina and by Research Contract N° 2816/R1/R2 of the International Atomic Energy Agency. The authors wish to thank Lic. Alba Zaretzky de Saleh for her assistance with the statistical analysis. The authors wish to acknowledge the technical assistance of Miss Ofelia Chiesa and Miss Patricia Mandalunis.

References

1. Orcutt JA. (1949) The toxicology of compounds of uranium following applications to the skin. In: *Pharmacology and toxicology of uranium compounds*. Vol. 1. Chapter VIII, McGraw-Hill, New York.
2. Harris, WB. (1961) The experimental clearance of uranium dust from the human body. In *Inhaled Particles and Vapours* (C. N.Darries Ed.) pp. 209–215. Pergamon, London.
3. Hursh JB, Neuman WF, Toribara T, Wilson H & Waterhouse C. (1969) Oral ingestion of uranium by man. *Health Phys* **17**, 619–621.
4. Hursh JB & Spoor NL. (1973) Data on man. In: *Handbook of Experimental Pharmacology (Uranium, Plutonium, Transplutonic Elements)* (H. C. Hodge, J. N. Stannard & J. B. Hursh, Eds.), p. 167. Springer-Verlag, New York.
5. Haley DP. (1982) Morphologic changes in uranyl nitrate induced acute renal failure in saline- and water-drinking rats. *Lab Invest* **46**, 196–208.
6. de Rey BM, Lanfranchi HE & Cabrini RL. (1983) Percutaneous absorption of uranium compounds. *Env Res* **30**, 480–491.
7. Neuman WF, Fleming RW, Dounce AL, Carlson AB, O'Leary J & Mulryan B. (1948) The

distribution and excretion of injected uranium. *J Biol Chem* **173**, 737–748.
8. Neuman WF, Neuman MW & Mulryan BJ. (1948) The deposition of uranium in bone. I. Animal studies. *J Biol Chem* **173**, 705–709.
9. Adams N & Spoor NL. (1974) Kidney and bone retention functions in the human metabolism of uranium. *Phys Med Biol* **19**, 460–471.
10. Neuman MW & Neuman WF. (1948) The deposition of uranium in bone. II. Radioautographic studies. *J Biol Chem* **173**, 711–716.
11. Rowland RE & Farnham JE. (1969) The deposition of uranium in bone. *Health Phys* **17**, 139–144.
12. Stevens W, Bruegner FW, Atherton DR, Smith JM & Taylor GM (1980) The distribution and retention of hexavalent 233 U in the Beagle. *Rad Res* **83**, 109–125.
13. Picer M & Strohol P. (1968) Determination of thorium and uranium in biological materials. *Anal Chim Acta* **40**, 131–136.
14. Guglielmotti MB, Ubios AM, de Rey BM & Cabrini RL. (1983) Effects of acute intoxication with uranyl nitrate on bone formation. *Experientia* **40**, 474–476.
15. Itioz ME, Carranza FA (Jr.) & Cabrini RL. (1963) Histologic and histometric study of experimental occlusal trauma in rats. *J Periodont* **34**, 305–314.
16. López Otero R, Carranza FA (Jr.) & Cabrini RL. (1967) Histometric study of age changes in interradicular bone of Wistar rats. *J Period Res* **2**, 40–45.
17. Carranza FA (Jr.) & Cabrini RL. (1967) Histometric studies of periodontal tissues. *Periodontics* **5**, 308–312.
18. Weibel ER & Elias H. (1967) Introduction to stereology and morphology. In: *Quantitative Methods in Morphology*, pp. 3–19. Springer-Verlag, Berlin.
19. Parodi RJ, Ubios AM, Mayo J & Cabrini RL. (1973) Total body irradiation effects on the bone resorption mechanism in rats subjected to orthodontic movements. *J Oral Pathol* **2**, 1–6.
20. Guglielmotti MB & Ubios AM (1982) Histometric study of extraction wound healing in rats. *J Dent Res* **61**, 615 (Abstr.).
21. Mann HB & Whitney DR. (1947) On a test of whether one of two random variables is stochastically larger than the other. *Ann Math Statistics* **18**, 50–60.
22. Pietrokowski J & Massler M. (1967) Ridge remodelling after tooth extraction in rats. *J Dent Res* **46**, 222–231.
23. Johansen JR. (1970) Repair of the post-extraction alveolus in the Wistar rat. *Acta Odonol Scand* **28**, 441–461.
24. Walinder G, Hammarstron L & Billaudelle V (1967) Incorporation of uranium. I. Distribution of the intravenously and intraperitoneally injected uranium. *Br J Ind Med* **24**, 305–312.

Address:
Dr. Angela M. Ubios,
Departamento de Radiobiología,
Comisión Nacional de Energía Atómica,
Avda. del Libertador 8250,
1429 Buenos Aires,
Argentina.