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An uncomplicated method for making periodical identical radiographs of experimental bone lesions in the rat. A technical report.

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Introduction
It is often desirable to study a disease longitudinally in its different stages in each individual animal. This may reduce the number of animals needed for the study, which means less time spent in repetition of long series, less money spent on breeding, purchase and care of animals, and less loss of time and money if an experiment should prove to have been in vain.
If each individual animal is monitored continuously, it may also be possible to discontinue failed experiments at an earlier point in time, thus freeing resources and perhaps avoiding unnecessary stress for the animals. If smaller series of animals are used, it may be possible to a larger extent to confine an experiment to siblings or litter mates, thus limiting the biological variation in an experiment.
In bone studies, the use of rats in developing new methods for studying arthrosis/arthritis (*Drelon et al* 1993, *Nakamuraet et al* 1991, *Drelon et al* 1992) or other pathologies (*Wang et Stashenko* 1991, *Hull et al* 1990, *Lüllmann-Rauch et Schleicher* 1992, *Phillipartet al* 1994, *Lekkas* 1994), operation methods (*Segal et al* 1991, *Romana et Masquelet* 1990, *Alsawaf et al* 1991, *Neufeld* 1992, *Wang et al* 1994, *Bluhm et Laskin* 1995), or bone healing as a separate entity (*Hämälainen et al* 1990, *Mueller et al* 1991, *Minkowitz et al* 1991, *Isaksson et al* 1993) is very common. When hard tissues are studied, radiology in one form or another is mandatory.Today, radiology is rapidly becoming computerised, opening up possibilities in image analysis hitherto feasible only at great cost and labour. The use of serial, identical radiographs is one such example.

The aim of this study was to find out if it is possible to expose serially reproducible radiographs on live rats over a period of time, using a removable stent.

Materials and Methods
The Sens-A-Ray™ system for direct digital radiography was used for this new method. Direct digital radiography eliminates the need for development and allows instant image processing and analysis (*Welander et al 1993*). The Sens-A-Ray™ system has technical properties on level with and sometimes surpassing those of E-speed film (*Welander et al* 1993, *Welander et al* 1994a, *Welander et al* 1995).
A model developed for the study of hematogenous osteomyelitis in the Wistar rat was used in the study (*Hienz et al* 1995). The study was approved by the Swedish animal ethics committee (Stockholms Södra Försöksdjursetiska nämnd). The rats were housed in the Animal Department of Huddinge University Hospital and cared for by their professional staff.
Two different sites were chosen to study, the mandible, and the tibia. The surgical procedure is described entirely in Hienz et al (1995), but as a précis, female Wistar rats (BK Universal, Sollentuna, Sweden), weighing 185 grams at start of study were used. The rats were anaesthetised using first 1.8 ml 6% chloral hydrate, which was injected i.p., and then 0.1 ml of Hypnorm™ (fluanison 10mg/ml and fentanyl 0.2 mg/ml, Janssen Pharmaceutical, Beerse, Belgium) injected i.m. An incision was made over the right mandible and the ramus was dissected. A hole was drilled in the ramus, and 50µ of 5% sodium morrhuate was

injected, after which the incision was sutured. The femur was treated in the same fashion.
To produce the osteomyelitis, the rats were injected with 1.0 ml of 5 x 10^7 CFU of *Streptococcus aureus*, of the strain Phillips, in the femoral vein. Controls were injected i.v. with saline.
An impression of the anterior portion of the upper jaw, including the incisiors, was made with Swedon™ acrylic resin, into which a stiff orthodontic wire had been incorporated. In a holder for the Sens-A-Ray™ detector the loose ends of the orthodontic wire were fixed in the holes meant for a Rinn™ positioner, in such a fashion that the rat's head was correctly positioned (Fig. 1). The stent could easily be inserted and removed, and fitted all the rats, which were the same age and size.

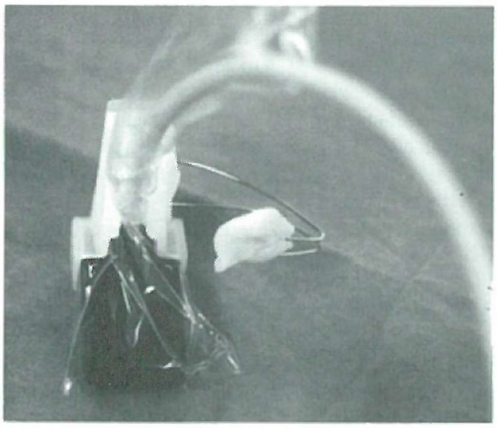


Fig 1. The stent, produced by direct method, in the same manner as a dental impression, using cold-cure acrylic resin (Swedon™), and stiff orthodontic wire.

After the stent was fixed to the anterior rat palate, the rat was positioned. Three different projections were used. First, the rat was positioned on its side (Fig 3 a, b) and a cephalogram made. Secondly, the rat was placed on its abdomen, and at right angle to the positioning stent. (Fig 2a, b). This radiograph is similar to the lateral oblique projection. When tibia radiographs were made, the rat was placed by eye only. (Fig 4a, b). Care was taken that the rat had received an adequate dose of Hypnorm™ to ensure complete muscle relaxation. Radiographs were taken every 7 days. On all occasions except during the surgery, the rats were anaesthetised with Hypnorm™ only.

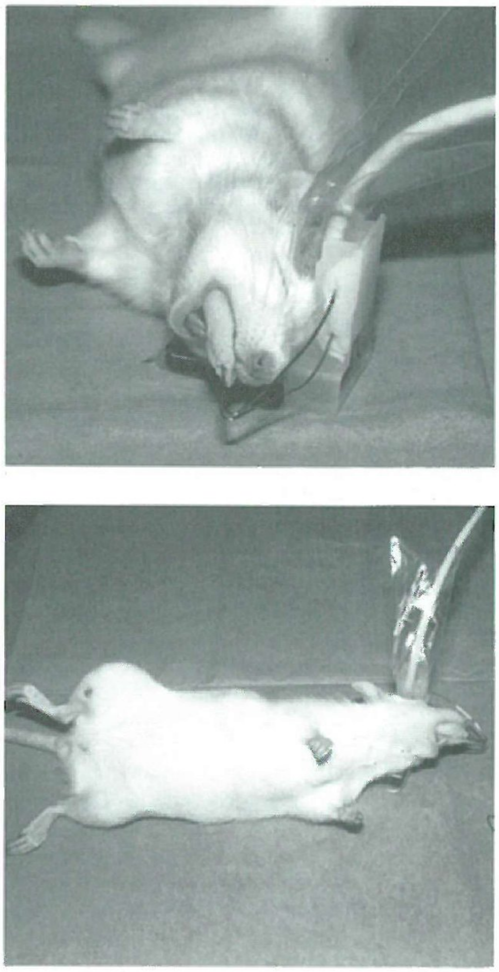
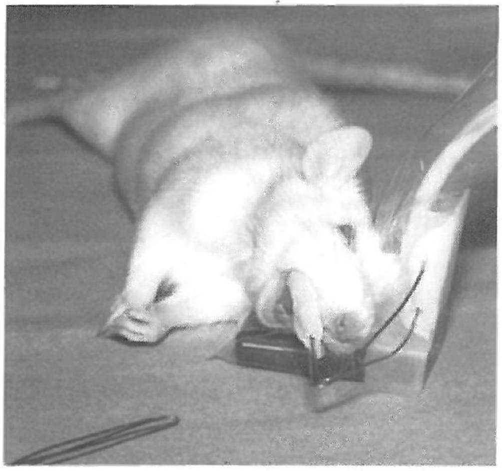


Fig 2a, b. Rat positioned on its side, making a radiograph possible, akin to a cephalogram.

A Siemens x-ray machine with Bi 125/30/50R tubes and 1 mm Al-filter was used. The settings were 120 cm "film" - focus distance, 60 kV, and 10 mAs for the mandible, 8 mAs for the tibia.
Twelve weeks after the operation, the largest of the rats could no longer be fitted into the stent, and they were sacrificed with an overdose of chloral hydrate injected ip.



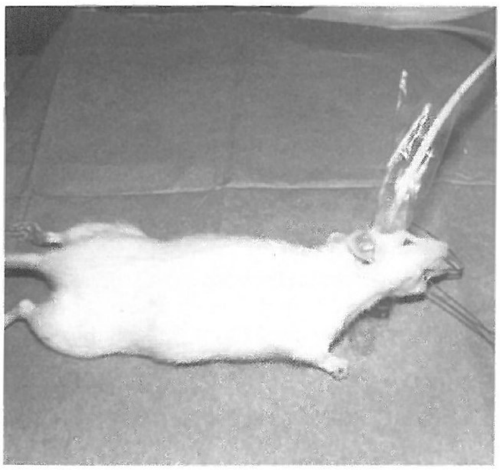


Fig 3a, b. Rat positioned on its abdomen, and at right angles to the positioning stent, producing a radiograph like a lateral oblique projection.

Results

It proved relatively simple to position the rats, provided the rats were adequately anesthesized to ensure muscle relaxation. In the cephalometric series shown, it is possible to follow the development of bony change in the rat mandible (Figures 5a-d & 5e-f). Figures 6a & b are examples of typical oblique lateral and tibia radiographs.

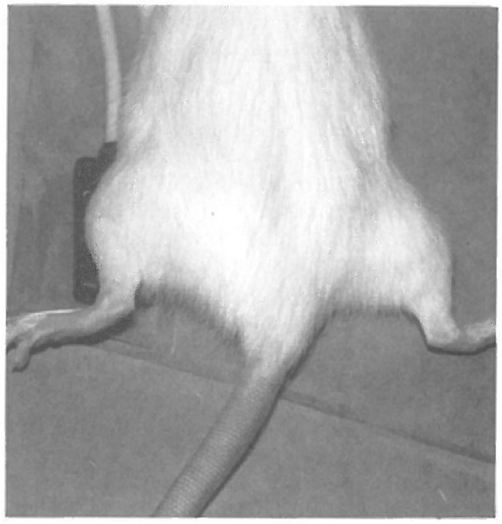


Fig 4. Rat positioned for tibia radiograph.

Discussion

This study shows that it is possible to make a series of reproducible radiographs which allows one to follow bony changes in the same individual experimental animal. Wang & Stashenko (1991) analysed periapical lesions on rat molars on digitised radiographs, but these radiographs were made after the rats had been killed, and were thus not reproducible.

Hull et al (1990) described the difficulty of following serially the induction and growth of murine colon cancer and the difficulty of assessing therapeutic manipulations without killing the animals. They demonstrated that it is possible to use another digital radiographic technique, fluoroscopy, with a sensitivity as good as total colonoscopy in identifying colon lesions in rats.

In assessing the use of visible light curing resin for vertebral body replacement, Segal et al (1991 and Alsawaf et al (1991) made radiomicrographs of the rat spine as the rats were sacrificed at 2, 4 or 6 months post operatively after implant surgery, but no serial radiography was attempted. Minkowitz et al (1991) made repeated radiographs on the rat femur fixed between polyethylene plates in order to study callus formation. Sweeney et al (1995) in studying the repair of rat calvarial defects using extra cellular matrix protein gels, used multiple

CT scans in order to determine bone repair. This is an efficient but expensive method. Conventional radiography has the inherent weakness of dark-room procedures, which are time consuming. During this time, the lightly anaesthetised rat could wake up, which would make retakes difficult, as further injections would be necessary, which could result in rats lost before the end of the experiment. Direct digital radiography eliminates the need for development and allows instant image processing and analysis (*Welander et al* 1993), and make instant retakes possible, should they prove necessary. The Sens-A-Ray™ system for direct digital radiography was chosen for this new method, since it has technical properties on level with and sometimes surpassing those of E-speed film (*Welander et al* 1993, 1994a, 1995).

Direct digital radiography is currently developing very rapidly, with many different systems available on the market. Although more expensive in the initial purchase than the conventional film based system, direct digital radiography is economical over a period of time, and well within the budget of most research laboratories.

Digital radiography also opens the possibility of subtraction or colour coding (*Welander et al* 1994b), which is ordinarily only possible in a radiology department, because of considerable costs in time, skills and equipment.

Even if these possibilities are not exploited, the advantages of serial radiographs, depicting the same lesion or anatomical structure in the same rat over a period of time are obvious. Although no effort was made in this study to analyse the bony changes seen, the discrete changes from week to week are easy to observe. It is common to determine as a criteria that a certain size of lesion must be obtained before a study is concluded. This can be ascertained radiologically without sacrificing any rat in advance. Recurrences and slower healing than expected need not make it necessary to to start a project over, because a too speedy sacrifice had taken place.

One of the radiographic projections produced in this study was a cephalogram. Orthodontic researchers do tracings of rat mandibles, similar to those performed as treatment planning on human patients (*Monje et al* 1993, *Brin et al* 1990). If these radiographs were in a digital form, no doubt computer programs intended for humans could be adapted and time consuming routine work avoided.

Every part of the rats anatomy should be possible to examine with direct digital radiology, since contrast media can improve soft tissues visibility even more. Contrast radiography has been performed successfully on the murine colon (*Hull et al* 1990).

The rats in this study were anaesthetised with Hypnorm™ (fluanison 10mg/ml and fentany l0.2 mg/ml), which was injected in the gluteus maximus muscle. The rats tolerated the weekly injections very well, and no rat was lost because of the frequent anaesthesia. The dose was 0.1 ml at onset, but it was necessary to increase the dose as the rats grew, and more than their weight increase indicated, so possibly the rats developed a certain resistance to Hypnorm™.

As a conclusion, using simple methods and advanced technology in combination, it is possible to produce serial radiographs of great similarity of the rat mandible and tibia. Because of this, this technique has the potential to save animals and resources such as time, money and space. All the advantages of image processing are available, relieving the scientist of tedious routine work.

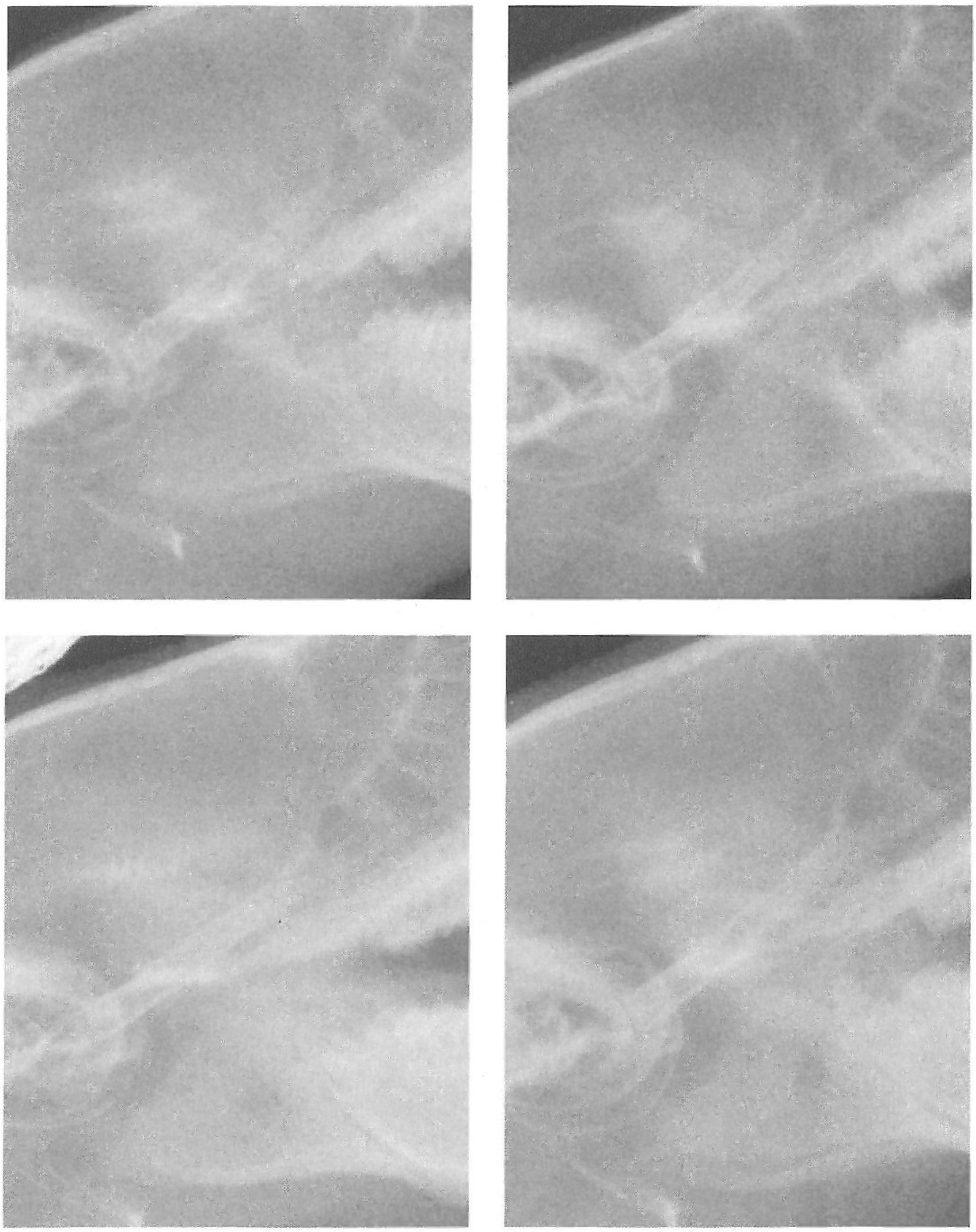
Summary

An uncomplicated method for making periodically similar radiographs of the rat mandible is presented. A stent was produced by making an impression of the rat maxilla using an acrylic resin, incorporating an orthodontic wire, and fixing the impression to a Rinn™ holder for the detector of the Sens-A-Ray™ direct digital radiographic system. This inflexible stent was then inserted and fixed to the maxilla of rats, anaesthetised with Hypnorm™ (fluanison 10mg/ml and fentanyl 0.2 mg/ml). In this fashion, serial radiographs were taken once a week for 12 weeks, making it possible to follow the healing of experimental bone lesions.

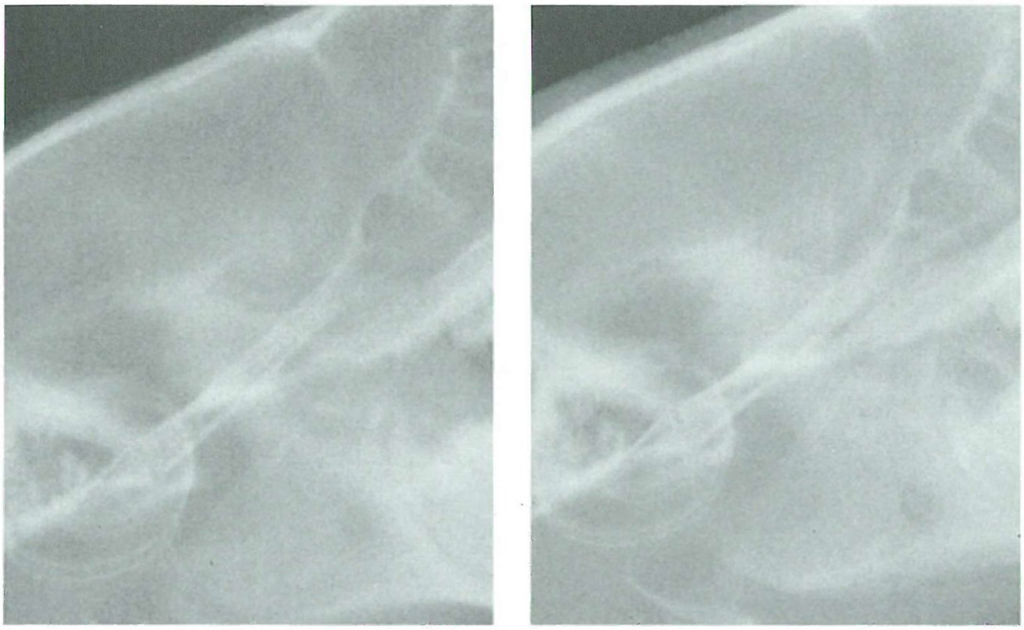
Sammanfattning

En okomplicerad metod för att framställa periodiskt lika röntgenbilder av bl.a. råttmandibel presenteras. En stent framställdes genom att avtryck togs av råttmandibeln med självpolymeriserande akrylat. I detta akrylat införlivades under stelnandet ortodontisk ståltråd, och stenten fixera vid en

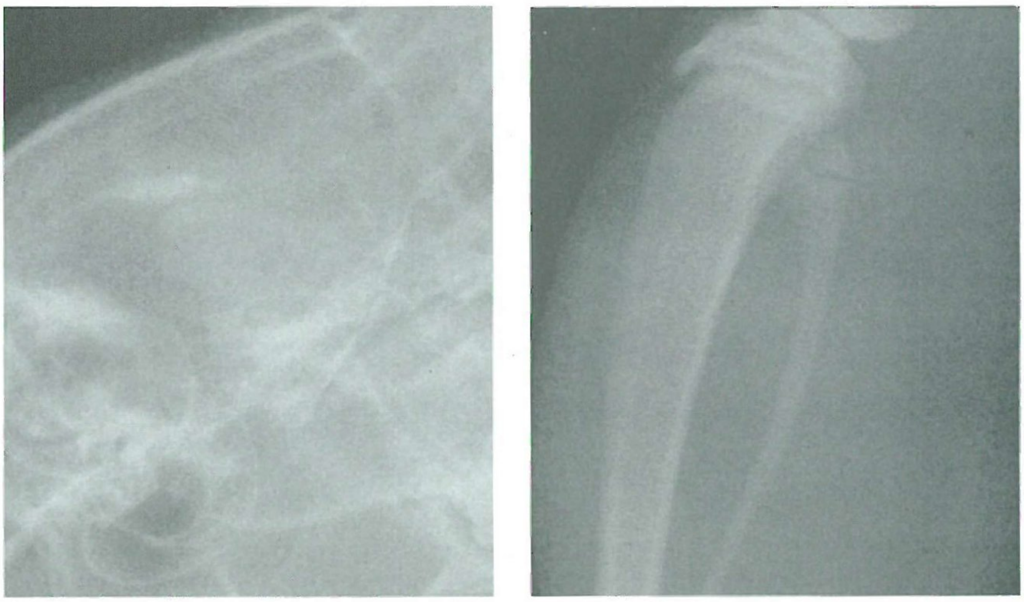
Figures 5a-d. Examples of cephalograms preoperatively and at 1, 4, and 6 weeks



Figures 5 e & f. Examples of cephalograms at 9 & 11 weeks



Figures 6a & b. Examples of oblique lateral and tibia radiographs



Rinn™ hållare til Sens-A-Ray™ systemet för direkt digitala röntgenbilder. Denna styva stent insattes i munnen på råttor, som anestiserats med Hypnorm™ (fluanison 10mg/ml and fentanyl 0,2 mg/ml). På detta vis kunde röntgenbilder i serier tas under 12 veckor, under vilken tid experimentella benlesioner följdes.

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