Acta Radiologica Oncology 25 (1986) Fasc. 1

FROM THE LABORATORY OF EXPERIMENTAL BIOLOGY, DEPARTMENT OF ANATOMY, THE ENT DEPARTMENTS OF THE HOSPITALS IN MÖLNDAL AND GOTHENBURG, THE INSTITUTE FOR APPLIED BIOTECHNOLOGY, AND THE DEPARTMENT OF CLINICAL ONCOLOGY AND RADIATION THERAPY, UNIVERSITY OF GOTHENBURG, S-400 33 GOTHENBURG, SWEDEN.

PROVOKED REPETITIVE HEALING OF MATURE BONE TISSUE FOLLOWING IRRADIATION

A quantitative investigation

M. JACOBSSON, P. KÄLEBO, T. ALBREKTSSON and I. TURESSON

Abstract

A titanium implant, the bone harvest chamber (BHC), was used to investigate the regenerative capacity of mature bone after irradiation. One BHC was inserted in each proximal tibial metaphysis of a rabbit. One of these implant sites was irradiated (^{60}Co single dose) to either 15 or 25 Gy while the other served as control. Newly formed bone grew through a canal that penetrated the implant. This newly formed bone was harvested from the implant every three weeks following irradiation and then quantified by microradiography and computer-assisted densitometry. In this way a ratio between bone formed on the irradiated side in comparison with the control could be established. An immediate depression in bone formation compared with the non-irradiated controls, was seen at both dose levels. A recovery in bone regenerative capacity was seen at 15 weeks after 15 Gy while the decrease in bone formation remained constant after 25 Gy during the 30 week follow-up period.

Key words: Radiation biology; rabbits, irradiation, bone healing.

The effect of irradiation on osteogenesis has been studied by many authors and it is well known that irradiation doses in excess of 10 Gy result in impaired fracture healing (8, 13). JACOBSSON et coll. (4) have demonstrated that the regenerative capacity of bone is much restored after 15 Gy ^{60}Co irradiation. It was found that bone healing improved by a factor of almost 2.5 one year after irradiation as compared with 4 weeks.

To investigate the background of post-irradiation impairment of osteogenesis, an experimental situation was constructed where the irradiated bone tissue could be provoked to form new bone at regular intervals after irradiation. This was accomplished by the use of the bone harvest chamber (BHC) (1), which is a titanium implant that was inserted into the proximal tibial metaphysis of the rabbit. Newly formed bone grew through a canal that penetrates the implant. By removing a lid from the BHC, it was possible to harvest this newly formed bone without sacrificing the animal. Such harvesting was then performed regularly at 3 week intervals over a follow-up period of up to 30 weeks. The harvested tissue was analysed by microradiography and computer-assisted microdensitometry to allow for a quantitative estimation of the amount of bone formed in the irradiated tibia and the control tibia of the same animal. In the present investigation the animals were subjected to single doses of either 15 Gy or 25 Gy.

Materials and Methods

Fourteen adult lop-eared rabbits between 10 and 14 months of age and of both sexes were used for the experiment. They had all closed epiphyseal plates as evidenced by radiography.

The bone harvest chamber (BHC) consists of a 6 mm wide and 10 mm high cylindrical framework and a removable center-piece with two attachment screws. There are two 1 mm wide holes in the framework so located that they together with an archlike passage in the bottom of the center-piece form a continuous canal when the implant is assembled. Bone growth will occur into this canal and is completed in 3 weeks after insertion of the BHC

Accepted for publication 30 September 1985.

into the rabbit proximal tibial metaphysis or 3 weeks after a harvest (Fig. 1).

Surgical procedure. The animals were under general anaesthesia using intramuscular administration of Hypnorm (Mekos, Helsingborg, Sweden) at a dose of 0.7 ml per kg body weight and hour and intraperitoneal administration of Valium (Roche) at a dose of 0.5 ml per kg body weight and surgery performed under aseptic conditions. The skin and fascia over the medial side of the proximal metaphysis were opened via separate, curved incisions. The periosteum was then removed via a circular incision 8 mm in diameter. Using a trephine of 5.5 mm diameter the medial cortex was removed under profuse irrigation with saline solution (0.9 mg/ml) to minimize heat induced damage to the implantation site. The BHC was then screwed into the hole thus made. Each animal had two implants inserted, one in each proximal tibial metaphysis. One tibial metaphysis was irradiated with the contralateral side serving as a control; each animal thus served as its own control.

Irradiation procedure. The rabbits were irradiated at a temperature of 22°C. During the irradiation the bone of the animal was placed upon a 10 cm thick polystyrene phantom. ^{60}Co gamma irradiation was chosen to minimize the difference in the absorbed dose in soft tissue and bone and in the disturbances in the radiation field due to the titanium chamber. Source skin distance was 60 cm and the field size was 7.5 cm × 7.5 cm. The dose rate was about 1 Gy/min. A 5 mm bolus was applied to ensure full build-up. The absorbed doses quoted below refer to the absorbed dose in water at the reference depth of 5 mm.

The rabbits were divided into two groups, each with 7 animals. The animals of group A received a single dose of 15 Gy to one tibial metaphysis, the other metaphysis serving as a control. The animals of group B were irradiated in a similar fashion with a single dose of 25 Gy.

Harvest procedure. The first harvest was performed 3 weeks after implant insertion, on the same day but before irradition was given. This first harvest only served as an indication of satisfactory incorporation of the implant and was not included in the material. Every 3 weeks thereafter a harvest was performed until animal sacrifice at pre-determined intervals 6 to 30 weeks after implant insertion.

In order to perform the harvest the animals were anaesthetized with Hypnorm and Valium. After skin and fascia incisions the center-piece was lifted out of the implant following unscrewing of the attachment screws (Fig. 2). The bone that had invaded the implant was cylindrical in shape and of 1 mm diameter and 4 mm length in normal, non-irradiated animals. After removal of the newly formed tissue from test and control, the center-piece was again put back into the framework of the BHC and attached by the two screws. Fascia and skin incisions were then carefully sutured. The whole harvest operation was performed under aseptic conditions. The newly formed bone was after removal immediately put into a buffered (pH 7) formalin solution.

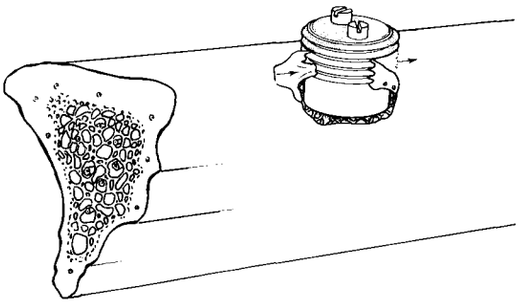


Fig. 1. The harvest chamber is a titanium implant with a penetrating canal. After insertion of the chamber in the proximal, tibial metaphysis of the rabbit, bone and vessels will grow through this canal.

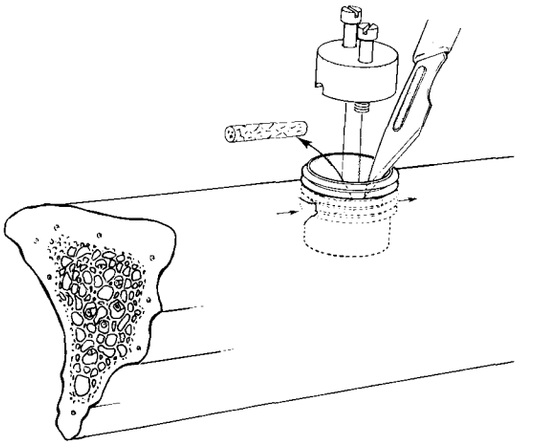
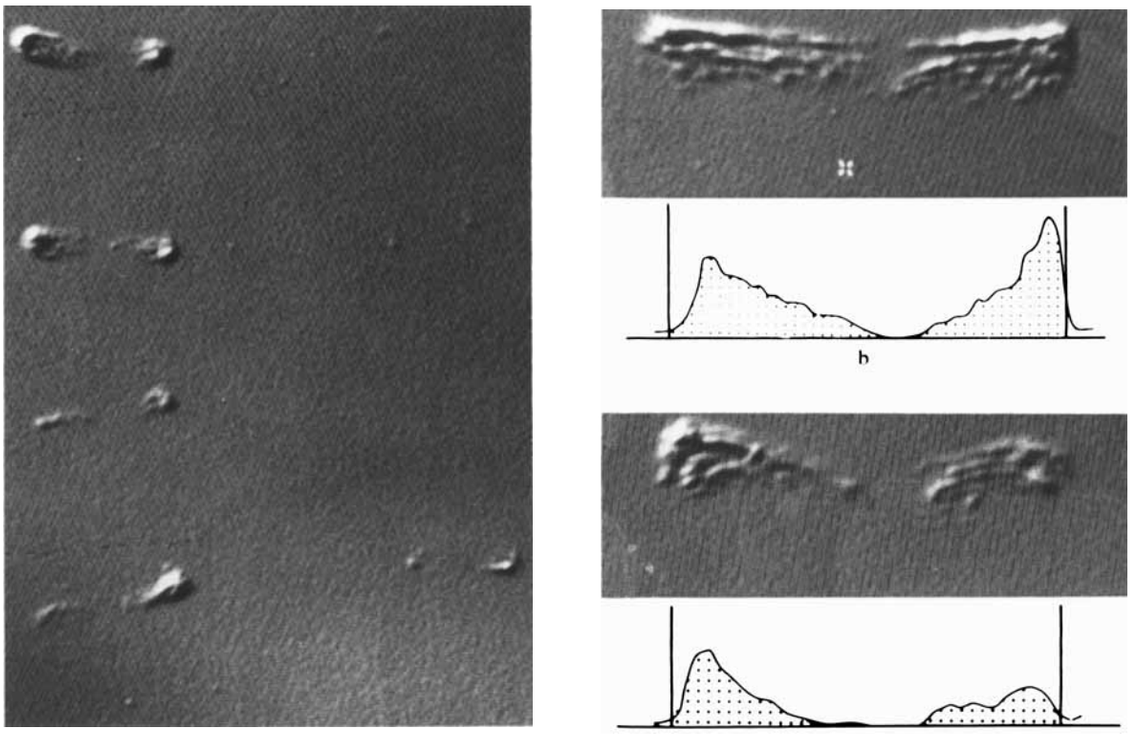


Fig. 2. Three weeks after chamber insertion, with the animal in light anaesthesia, the skin over the implant is opened and the center-piece of the chamber lifted out. It is now possible to perform a harvesting of the bone that has grown into the implant without sacrificing the animal. One bone specimen is collected from the test implant inserted in one tibial metaphysis and another from the control in the contralateral extremity. The lid is then replaced and the skin sutured over the implant until the next harvest another 3 weeks later. The bone that is collected at the harvests is microradiographed and the bone volume is assessed with a computer-assisted technique.

Microradiography. The tissue in the canal of the implant was put in direct contact with Kodak high resolution glass plates of type 1A. Microradiography was then performed at 17.5 kV and 20 mA with the use of a Machlett-type roentgen tube, type OEG-50. All specimens of the present study were microradiographed simultaneously to guarantee identical conditions.

Microdensitometry. The content of bone mineral substance was assessed from the microradiographic plates by the densitometric method of Buch et coll. (3), briefly described as follows:



a c

Fig. 3. a) Microradiographic image showing four consecutive harvests from the same animal. On the left pseudoplastic images of the controls indicate a substantial bone formation. As can be seen on the right side, hardly any bone invaded the chamber after irradiation to 25 Gy at the harvests after 6, 9 and 12 weeks. After 15 weeks (lower right) there is some bone formed on the test side. b, c) Two control specimens with the dotted area representing the bone volume calculated by the computer. In this case the amount of bone that grew into the irradiated controls (15 Gy) was negligible.

The transmittance of light through a microradiographic plate varies locally with the photographic density of the plate. Under rather general conditions it can be shown that the logarithm of the local transmittance varies directly with the weight of absorbing substance contributing to the local density in the radiographic plate (6). Thus integration of the logarithm of light transmittance over the surface of the plate (i.e. summing the contributions to light transmission all over the plate) will yield the total amount of bone mineral substance expressed in arbitrary mass units.

The microdensitometric measurements and calculations were carried out by an interactive image analysis system (Kontron IBAS I and II), using a television camera to translate the radiographic image into electric signals. The microradiograms pertaining to the test and control sides of an animal were evaluated using the same setting of the television camera (Fig. 3). The amount of bone formed in the implant of the irradiated side was compared with that of the contralateral control by means of the formula:

$$\text{Alteration (\%)} = -\left(100\left(1-\frac{\text{test value}}{\text{control value}}\right)\right)$$

Histology. After microradiography, the tissue specimens were again put into a 4% neutral formalin solution and decalcification was then accomplished using 30% formic acid. After embedding in paraffin 6 μm thick cross and longitudinal sections were cut and stained with haematoxylin-eosin.

Results

General inspection. All animals remained healthy throughout the observation period. None of the implants was loose at any of the harvest procedures.

Microdensitometry. Group A (15 Gy). There was an initial depression in bone regeneration. The average reduction in bone formation at the first harvest at 3 weeks after irradiation was 81.5 per cent as compared with non-irradiated controls, with a range from 35.3 to 100 per cent. The bone regenerative capacity increased with increasing time after irradiation. Already at 15 weeks after irradiation was there a clear tendency towards recovery of bone regeneration (Figs 4, 5, Table 1).

Group B (25 Gy). The initial depression in bone formation was considerable: 95.7 per cent (range 79.0–100%) for the group as a whole. There was no obvious trend towards recovery of bone regeneration during the 30 weeks following irradiation. At 15 weeks after irradiation

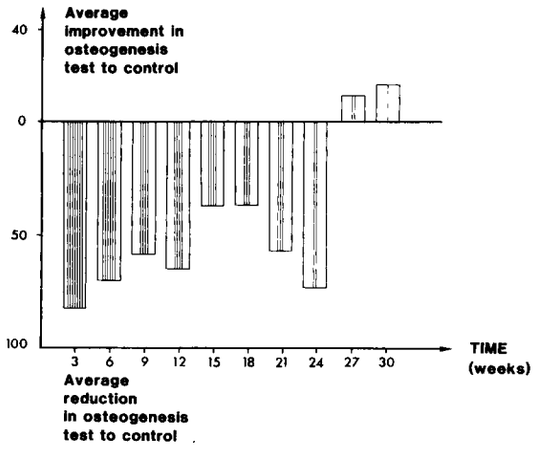


Fig. 4. Graph showing the change (in per cent) in bone formation as determined from the bone tissue specimens harvested every 3 weeks after irradition of 15 Gy. The results at each 3-week-interval represent the average depression in osteogenesis of the animals included in that interval. Each animal is represented by a thin vertical line within the larger vertical bar.

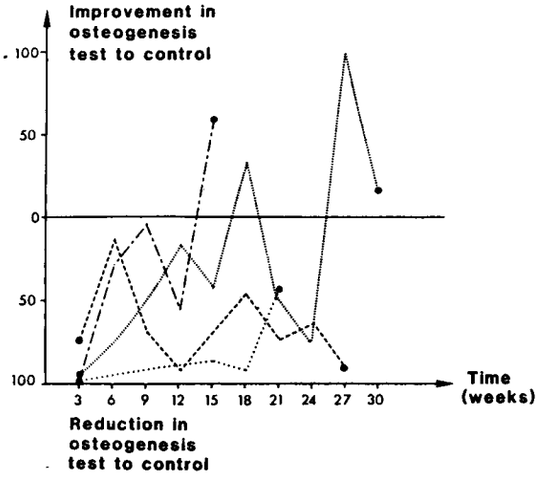


Fig. 5. Change (in per cent) in osteogenesis after irradiation of 15 Gy (single dose) shown individually from the 4 animals with the longest follow-up periods at this dose level. From an initial depression of the osteogenic capacity there is a clear tendency towards recovery at later stages.

Table 1

Volume of bone (test/control, units) formed in harvest chambers inserted in bone irradiated to 15 Gy

Animal No.	Harvest									
	1	2	3	4	5	6	7	8	9	10
1	213/553									
2	0/36	0/21								
3	311/481	0/498								
4	18/1106	557/779	768/808	341/796	474/298					
5	1/605	17/442	9/168	36/375	17/133	25/326	81/138			
6	35/169	143/164	79/345	46/656	112/493	189/354	53/250	58/177	32/455	
7	12/309	45/206	241/530	716/866	258/445	207/161	362/714	86/388	710/349	413/356

there was still a profound retardation of bone formation and the subsequent harvests did not show any significant recovery (Figs 6, 7, Table 2).

Histologic evaluation. The bone tissue formed in the canals of the irradiated implants had a retarded lamellarization and a bone tissue of a more fibrous character than seen in the controls. Apart from this no gross morphologic alterations were seen. The vasculature appeared normal with no signs of thrombus formation, increased vessel wall thickness or extravascular leakage of blood cells (Fig. 8).

Discussion

Several factors are responsible for an appropriate bone healing response. It seems like pre-existing osteoblasts that survive the trauma to some extent can contribute to fracture healing in form of the so called primary callus response (7). The quantitatively most important cellular source for bone repair is, however, the undifferentiated mesenchymal cells which are induced into preosteoblasts and, subsequently (through mitosis) into new bone-forming cells (7, 12, 14). Irradiation injury to bone probably will primarily affect the un-differentiated bone and mesenchymal cells even if a negative influence on the inductive substance of bone (the morphogenetic protein) cannot be ruled out (11).

In another investigation (1), it has been demonstrated that the bone regeneration, using the BHC methodology, varies from one harvest to another in animals that have not been subjected to irradiation. In those control cases, however, the bone forming capacity in the left and right leg, respectively, was very similar at the same harvest.

From the results of the present investigation it is evident that the animals that received 25 Gy had a greater initial depression of bone formation and a reduced capacity for recovery of bone regeneration during the 30 weeks

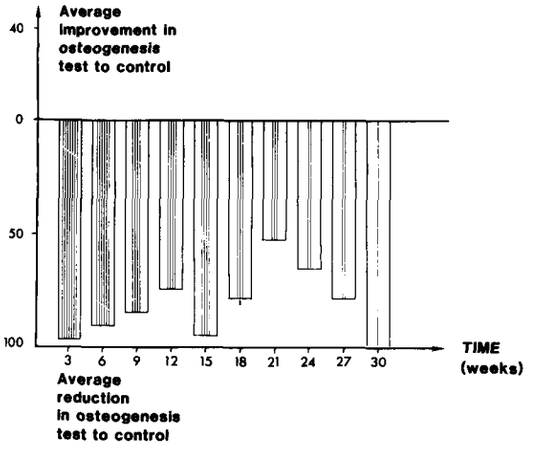


Fig. 6

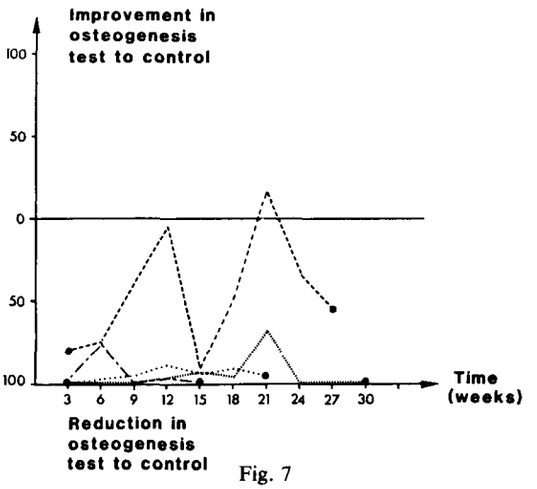


Fig. 7

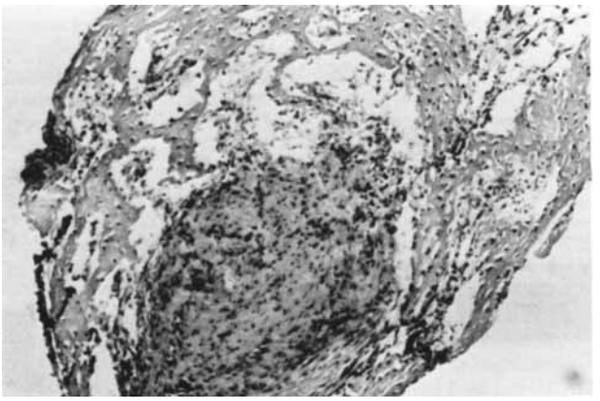


Fig. 8

Fig. 6. Graph showing the change (in per cent) in bone formation as determined from the bone tissue specimens harvested every 3 weeks after irradiation of 25 Gy. The results at each 3-week-interval represent the average depression in osteogenesis of the animals included in that interval. Each animal is represented by a thin vertical line within the larger vertical bar.

Fig. 7. Change (in per cent) in osteogenesis after irradiation of 25 Gy (single dose) for the 4 animals with the longest follow-up periods at this dose level. There is no tendency towards a general recovery of bone formation with longer times of follow-up.

Fig. 8. Histologic appearance of bone tissue formed in the canal of a bone harvest chamber 3 weeks after irradiation of 25 Gy single dose. The fibrous tissue in the center is not clearly demarcated from the bone tissue. A transitional zone between bone and connective tissue proper is thus created.

Table 2

Volume of bone (test/control, units) formed in harvest chambers inserted in bone irradiated to 25 Gy

Animal No.	Harvest									
	1	2	3	4	5	6	7	8	9	10
8	0/545									
9	0/69	9/764								
10	42/562	61/559								
11	13/1137	151/674	0/136	5/445	0/54					
12	0/311	38/1537	79/2076	99/1180	51/1201	61/1119	17/515			
13	118/562	139/550	358/568	511/549	67/465	273/468	309/270	231/323	92/204	
14	4/551	0/591	0/468	5/272	34/560	24/594	21/66	0/96	0/662	0/239

following irradiation than had the group that received 15 Gy. It is reasonable to assume that the dose of 25 Gy either kills the majority of the osteoprogenitor cells directly or causes a cellular damage which prevents normal differentiation to bone forming cells. At the 15 Gy dose level, on the other hand, it is probable that fewer cells die or are permanently injured by the irradiation as the repair with time is clearly greater than seen after irradiation to 25 Gy. In fact, it has been shown (4), that the recovery of bone formation over a 12-month-period following irradiation of 15 Gy occurs by a factor of almost 2.5.

In the clinic, single irradiation doses as great as 15 Gy are not used, as fractionated irradiation is advantageous. However, a single dose of 15 Gy corresponds approxi-

mately to a CRE of 15, i.e. a value which often is exceeded in clinical, fractionated radiation therapy (5, 10). We believe the present result to indicate that bone surgery should not be undertaken until a certain healing time of the tissues has been allowed. In fact, TONNA & PAVELEC (9) in an in vivo study in the rabbit tibia have even found single doses of around 8 Gy to result in inhibition of proliferation of periosteal and endosteal osteogenetic cells, a finding which points to the possibility of a harmful effect below the common therapeutic dose levels. The careful approach at implant insertion advocated by BRÅNEMARK et coll. (2), even suggests that repeated radiographic examinations of newly inserted implants should be avoided to ensure a maximum cellular contribution to repair in the delicate situation immediately after implantation. After low energy roentgen rays, in contrast to high energy ^{60}Co, there is definitely an interfacial increase of the absorbed radiation dose due to the dense metal structure of the implant.

Conclusions

1) The bone harvest chamber is a simple and reliable method to study provoked bone regeneration after irradiation.

2) At both 15 and 25 Gy there was an evident initial depression in bone formation.

3) With increasing time from irradiation the animals that received 15 Gy tended to recover much of their bone regenerative capacity whereas the 25 Gy group had a persistent low bone forming capacity.

ACKNOWLEDGEMENTS

This investigation was supported by grants from the Swedish Medical Research Council (B84-12P-6475-02 and B84-12X-6533-02), the King Gustaf V Jubilee Foundation, the Trygg-Hansa Research Fund, the Gothenburg Medical Society and Greta and Einar Asker's Foundation.

REFERENCES

1. ALBREKTSSON T., JACOBSSON M. and KÄLEBO P.: The harvest chamber. A newly developed implant for analysis of bone remodelling in situ. *In*: Biomaterials and biomechanics. Edited by P. Ducheyne, G. Van der Perre and A. E. Aubert. Elsevier Science Publishers, Amsterdam 1984.
2. BRÅNEMARK P-I., HANSSON B. O., ADELL R. et coll.: Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. Almqvist and Wiksell International, Stockholm 1977.
3. BUCH F., ALBREKTSSON T. and HERBST E. Direct current influence on bone formation in titanium implants. Biomaterials 5 (1984), 341.
4. JACOBSSON M., JÖNSSON A., ALBREKTSSON T. and TURESSON I.: Short and long term effects of irradiation on bone regeneration. Plast. Reconstr. Surg. 1985 (in press).
5. KIRK I., GRAY W. H. and WATSON E. R.: Cumulative radiation effect. Clin. Radiol. 22 (1971), 145.
6. LANTZ B. and STRID K.-G.: Contrast formation in fluoroscopic videodensitometry. II. A comparison between theoretically computed and experimentally measured contrast. Acta radiol. Diagnosis 14 (1973), 625.
7. MCKIBBIN B.: The biology of fracture healing in long bones. J. Bone Jt Surg. 60 B (1978), 150.
8. REGEN E. M. and WILKINS W. E.: The influence of roentgen irradiation on the rate of healing of fractures and the phosphatase activity of the callus of adult bone. J. Bone Jt Surg. 18 (1936), 69.
9. TONNA E. A. and PAVELEC M.: Changes in the proliferative activity of young and old mouse skeletal tissues following Co-60 whole-body irradiation. J. Gerontol. 25 (1970), 9.
10. TURESSON I.: Fractionation and dose rate in radiotherapy. A study of cumulative radiation effects. Thesis, University of Gothenburg 1978.
11. URIST M. R. and HERNANDEZ A.: Excitation transfer in bone. Deleterious effects of cobalt 60 radiation-sterilization of bank bone. Arch. Surg. 109 (1974), 486.
12. — and MCLEAN F. C.: Osteogenic potency and new bone formation by induction in transplants to the anterior chamber of the eye. J Bone Jt Surg. 34 A (1952), 443.
13. WOODARD H. Q.: The influence of X-rays on the healing of fractures. Hlth Phys. 19 (1970), 791.
14. YOUNG R. W.: Nuclein acids, protein synthesis and bone. Clin. Orthop. 26 (1963), 147.