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# Periosteal Bone Formation on Medullary Evacuation

## A Bone Formation Model

(Preliminary communication)

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ABSTRACT

**A periosteal bone formation model based on the collateral reaction of the periosteum to evacuation of the medullary cavity has been developed. With this experimental model and on the basis of the values obtained and the precise statistical findings made in this study, further investigations on the influence of pharmacological and hormonal factors on periosteal bone formation should be possible.**

## INTRODUCTION

Destruction of the bone marrow even without simultaneous fracture of the bone is followed by periosteal bone formaction (1, 2). Richany et al. (6) and Mital & Cohen (4) have suggested that the periosteal bone formation that is initiated experimentally by such bone marrow destruction could be used for evaluating the influence of a specific factor on the bone formation.

The reproducibility of such evaluations made hitherto must be regarded as unreliable, on account of several factors. During reaming of the medullary cavity there is a pressure rise (1). When an increase in pressure is produced inside a tubular bone the bone marrow can be forced into the intracortical canals and disturb the cortical circulation (1). In some cases such a pressure increase can also result in a subperiosteal accumulation of bone marrow, a so-called medullo-haematoma (7), which gives rise to subperiosteal bone formation varying in magnitude. Danckwardt-Lilliestrom et al. (3) have demonstrated that the disturbances of the intracortical circulation are very limited and, in addition, reproducible when intramedullary pressure increases are avoided by evacuating the medullary cavity by suction before and during reaming. The essential requirement that the periosteum is left mechanically completely unaffected. This can be fulfilled by the fact that no medullo-haematoma is formed and that no direct surgical procedure is made to the periost.

The aim of the present investigation was to determine whether, under certain conditions, this periosteal bone formation also can be sufficiently reproducible to be used as a bone formation model.

## MATERIAL AND METHODS

Thirty-four rabbits weighing 2.8–3.2 kg were used. They were adapted to the cages and laboratory diet for 1 week prior to the experiments. One tibia was treated operatively, while the other served as a control. At the operation, which was performed under nembutal anaesthesia, the medullary cavity was opened with a 2 mm drill immediately above the ankle joint. A polyethylene catheter with an internal diameter of 1 mm was introduced into the medullary cavity (Fig. 1 *a*) and with great care it was advanced slowly to the proximal metaphysis during strong, continuous suction. Via a longitudinal incision through the patellar ligament the precondylar surface of the tibia was exposed and the medullary cavity was opened. A 5 cm long, relatively soft bottle brush with a diameter of 5 mm was then inserted into the medullary cavity down to the distal metaphysis (Fig. 1 *b*), while at the same time physiological saline solution was allowed to drop continuously over the upper opening of the tibia, and the fluid was sucked through the medullary cavity. In this way the contents of the medullary cavity in the diaphysis of the tibia were removed without the occurrence of any intramedullary pressure increase.

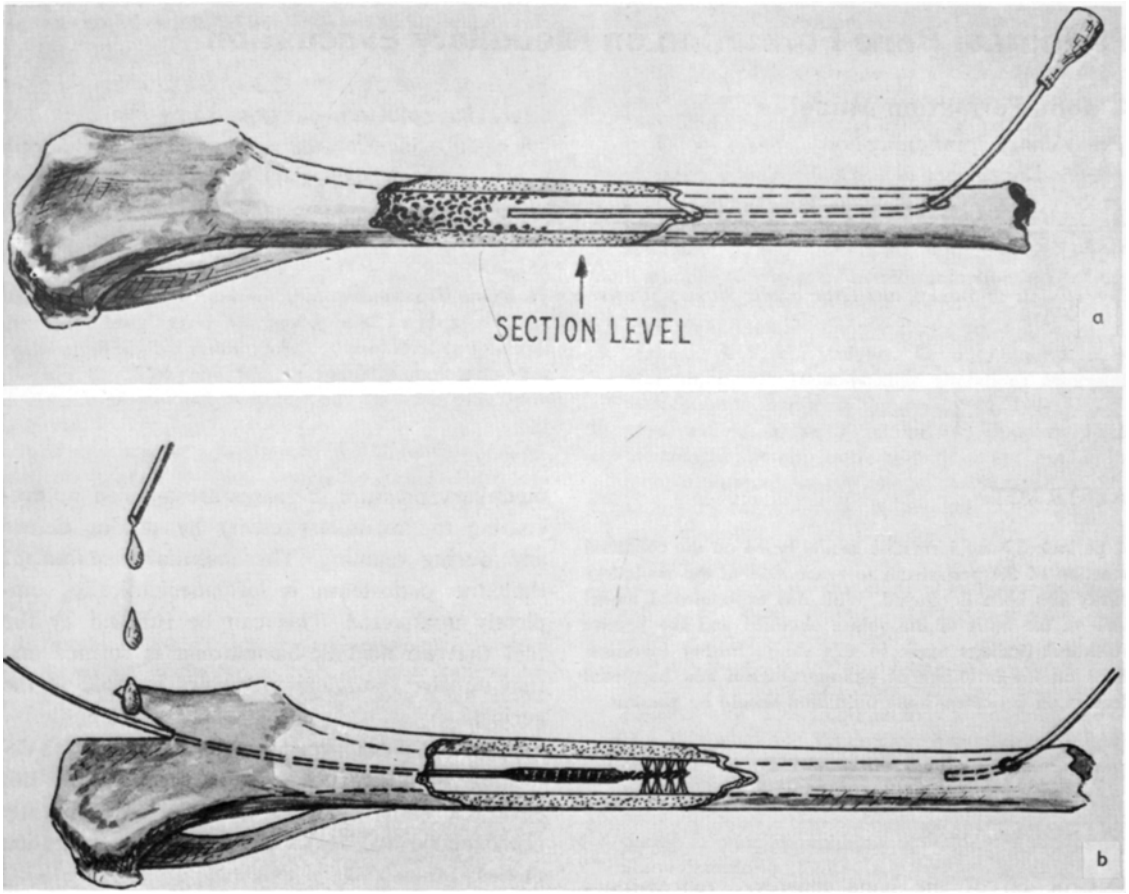


*Fig. 1.* Illustrations of the operative procedure on the rabbit's tibia. (*a*) Marrow evacuated through a distal hole with a suction catheter. (*b*) The brushing of the medullary cavity during continuous suction and saline constant dropping.

For bone labelling, oxytetracycline (terramycin, Pfizer) was given intravenously at the time of the operation. Other bonelabelling fluorochromes (5), viz. haematoporphyrine and DCAF,[1] were given on the 10th and 12th days, respectively, after the operation. The postoperative observation time was 14 days. Tibial preparations were freeze-dried and embedded in plastic. Cross sections (50–70 $\mu$) taken from the tibial diaphysis immediately below the tibiofibular synostosis (Fig. 1 *b*) on the treated side were compared with corresponding sections from the control side. The difference between the areas of newly formed periosteal bone on the treated and control sides were determined in surface area units for each animal by a method described by Danckwardt-Lilliestrom (1). The mean value for this difference (mean difference value) and the standard error was calculated for the whole group of animals.

[1] 2,4–bis *N*, *N'*–di–(carbomethyl) aminomethyl flouresence.

## RESULTS

Medullary evacuation in the 34 animals resulted in moderate periosteal bone formation which consisted mainly of primary osteones and circumferential lamellae. In a few animals fibrous bone formation occurred at the time of the second labelling. This bone formation was not considered to be a sequel of the marrow evacuation. Thus the animals with fibrous bone formation in the last part of the observation time have been excluded.

Representative pictures of the appearance of the cross sections are shown in Fig. 2 *a* and *b*, where *a* corresponds to the control side and *b* to the treated tibia. The inner periosteal labelling comprises terramycin labelling, while the outer labelling was produced by DCAF. The bone

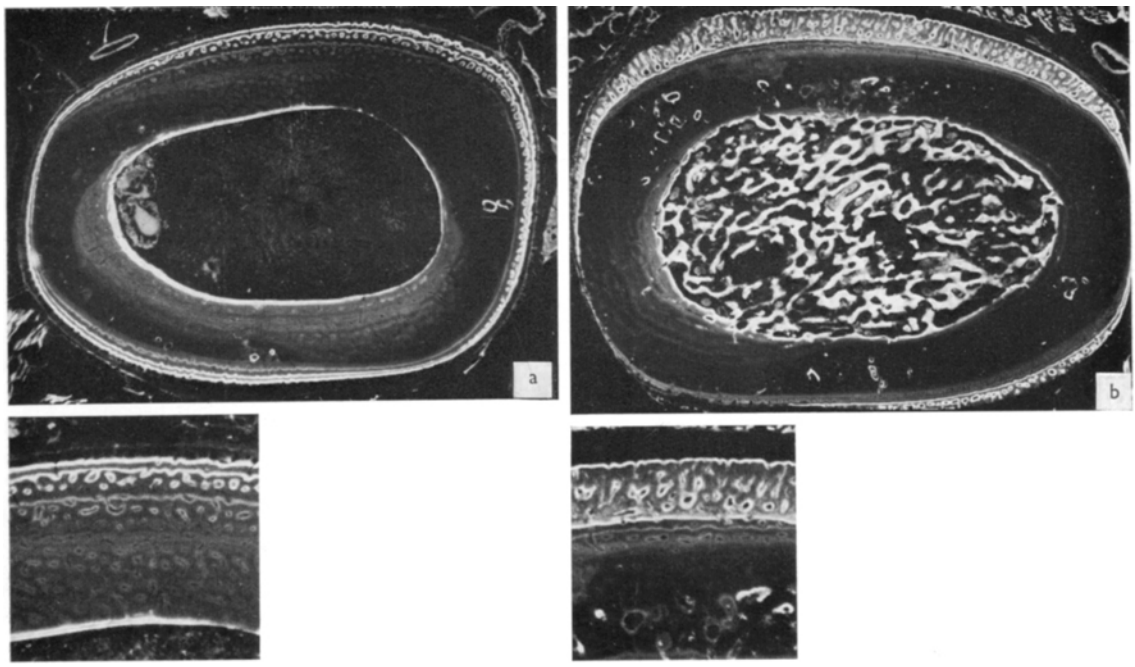


*Fig. 2.* Transversal sections of the rabbits tibia just below the fibular synostosis. The white linings represent the tetracycline labelling resp. DCAF labelling. (*a*) Right tibia indicates the periosteal growth when the marrow cavity is untouched. (*b*) Left tibia shows a rather large periosteal growth after the marrow evacuation.

formation between these two lines comprises the amount of bone formed during the experimental period. The difference in newly formed bone between the two tibiae in the individual animals can be seen in Table I.

A statistical analysis of the results from the 34 animals showed the mean difference value to be 18.19 surface area units. The standard error was 1.96. The difference between the treated and control tibia is *highly* significantly different from zero, i.e. the bone formation was highly significantly increased on the treated side compared with the control side. The standard error is very low, indicating a high confidence, in other words, a good reproducibility.

## DISCUSSION

Bone formation in fracture healing can change course depending on the influence of local and general factors. The local factors (periosteal damage, size of haematoma, vascular supply to the bone fragment, etc.) appear to have the greatest importance during the primary healing phase, while more general factors such as the endocrine environment and the general effect of trauma increase in significance during the later stage of the bone formation.

Up to now no methods have been described for differentiating and grading more exactly the influence of local (e.g. immobilization) and general but defined factors (drugs, hormones) on the periosteal bone formation. With the bone formation model suggested here the periosteum is subjected to standardized indirect influence which comprises, among other things, a considerable change of the blood circulation without any trauma to the periosteum itself. The treatment should be followed by proportional (standardized) periosteal bone formation which can be evaluated quantitatively.

Periosteal bone formation could be due to a mechanically weaker bone, caused by the operative procedure, but the uniform technic would guarantee similarity also in this respect between all the animals.

A criterion of a satisfactory result with this model is the finding of lamellar bone formation at the last labelling. With reference to the studies

Table I. *Amount lamellar periosteal bone formation*

| Rabbit No. | Left tibia (Medullary cavity evacuated) | Right tibia (Control) | Difference |
|---|---|---|---|
| 345 | 31.1449 | 4.0786 | 27.066 |
| 346 | 15.3569 | 5.2560 | 10.101 |
| 348 | 36.2122 | 9.2617 | 26.950 |
| 350 | 37.3892 | 8.4345 | 28.955 |
| 351 | 37.8544 | 8.4630 | 29.391 |
| 352 | 34.1974 | 7.5953 | 26.602 |
| 354 | 14.2876 | 8.8823 | 5.405 |
| 355 | 43.4337 | 5.9529 | 37.481 |
| 356 | 42.3860 | 12.6563 | 29.730 |
| 357 | 51.2925 | 13.4421 | 37.850 |
| 358 | 51.7180 | 22.5144 | 29.204 |
| 359 | 32.1349 | 13.9810 | 18.154 |
| 366 | 19.7768 | 10.0274 | 9.750 |
| 367 | 21.5794 | 11.7702 | 9.809 |
| 368 | 15.6593 | 8.6024 | 7.057 |
| 369 | 24.1023 | 5.5135 | 18.589 |
| 370 | 28.7098 | 7.9062 | 20.804 |
| 403 | 36.2194 | 11.7389 | 24.480 |
| 405 | 42.4190 | 9.0092 | 33.410 |
| 407 | 18.1726 | 11.7598 | 6.413 |
| 409 | 44.3622 | 9.9126 | 34.450 |
| 413 | 30.6305 | 21.0175 | 9.612 |
| 423 | 43.5373 | 18.0276 | 25.510 |
| 431 | 27.3375 | 9.1784 | 18.160 |
| 433 | 20.0030 | 8.3660 | 11.637 |
| 434 | 5.9210 | 3.9320 | 1.989 |
| 435 | 0.3747 | 0.4081 | −0.033 |
| 436 | 1.0869 | 1.0828 | 0.004 |
| 437 | 9.8560 | 6.2490 | 3.607 |
| 439 | 24.6530 | 9.0340 | 15.619 |
| 440 | 24.3920 | 7.1580 | 17.234 |
| 441 | 20.0110 | 10.9180 | 9.093 |
| 442 | 47.9240 | 14.7460 | 33.178 |
| 443 | 20.4590 | 14.8910 | 5.568 |
| | | | $M = 18.185$ |
| | | | S.E. = 1.963 |

of periosteal bone formation activity (1) we thus consider that fibrous bone formation as late as 12 days after evacuation of the medullary cavity, probably does not depend upon this procedure, but upon a later local trauma to the bone. Four animals with this deviation of bone formation pattern therefore have been excluded from our series.

The positive mean difference in amount of newly formed bone between treated and untreated tibiae must be regarded unequivocally as an effect of the operation. The amount of newly formed bone resulting from the medullary trauma must be considered to constitute the sum of the bone formation increase on the treated side and any bone formation inhibition on the untreated side.

It cannot be ruled out that the latter side is also affected by the trauma, a question which may be answered after studies of series with different treatment variables. The variable for evaluation chosen here (the difference value) can reveal and quantitate the influence of general factors on the bone formation. Such factors include variation in age, individual general growth differences, nutritional state etc. Since it can be assumed that the age factor is of very great importance for the size of the difference value, we have kept the age (weight) of the animals in this series within narrow limits. For the same reason we have attempted to obtain a standard basis as possible for the studies by giving the rabbits time to became adapted to the cages and the diet of the laboratory.

The mean value of the observations—the mean difference value (see "Results")—comprises a measure of the general effect of the marrow evacuation on the periosteal bone formation. Our aim is to be able to use the experimental model for studying the way in which defined factors (drugs, hormones and also trauma and inactivity) influence the bone formation induced by a standardized indirect effect on the periosteum.

It is considered that in such further studies the mean difference value from the different treatment groups can be tested for significance as compared with this basal value. Since it is aimed that the treatment variable will be the only factor distinguishing the treatment group from the basal group, a positive result of this significance test must give an unequivocal answer as to whether any effect has been exerted on the bone formation by the variable under study. Thus, this model gives a possibility of differentiating and grading generally or locally induced bone formation effects, though different in the time phase.

The ability of reproduction of the difference value, expressed as standard error, has such a good confidence, that it is probable that a substance or a procedure (plaster immobilization) that influences the periosteal bone formation, really will alter the mean difference value in a statistically significant grade.

## ACKNOWLEDGEMENTS

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