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INVESTIGATION OF THE CELLULAR RESPONSE TO FRACTURE ASSESSED BY AUTORADIOGRAPHY OF THE PERIOSTEUM

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The uptake of tritiated thymidine into synthesized DNA of cells in mitosis has been widely used for studying proliferating cells in many tissues. Cells which have incorporated labelled thymidine into their nuclei may be located in serial sections by autoradiographic methods (McLean & Urist 1961, Owens 1963, Tonna & Cronkite 1961).

By this means it is possible also to locate areas with proliferative activity, observe the migration of cells from one site to another, or estimate stages in the degree of differentiation. The periosteal surface is considered a relatively simple system for studying bone growth. In the resting bone the cells are situated in a fairly well-defined layer and exhibit long, slender, spindle-shaped, dark nuclei. The periosteal layer is discernible with fair certainty from the adjacent muscle layer, and it is delimited by the cortical membrane from the medullary, proliferating cells of the bone.

In the present study we employed the autoradiographic method for assessing the rate and spread of the proliferation induced in the periosteum of long bones after fracture. The object was to measure the increment of cells in the immediate vicinity of the fracture, along the length of the fractured bone and on the symmetrical, non-fractured bone, and also to determine the time of maximum cell proliferation after the fracture.

MATERIAL AND METHOD

The experimental animals were 44 five-week-old NMRI mice. Under ether anaesthesia a uniform bending fracture was induced by digital pressure on the mid-shaft of

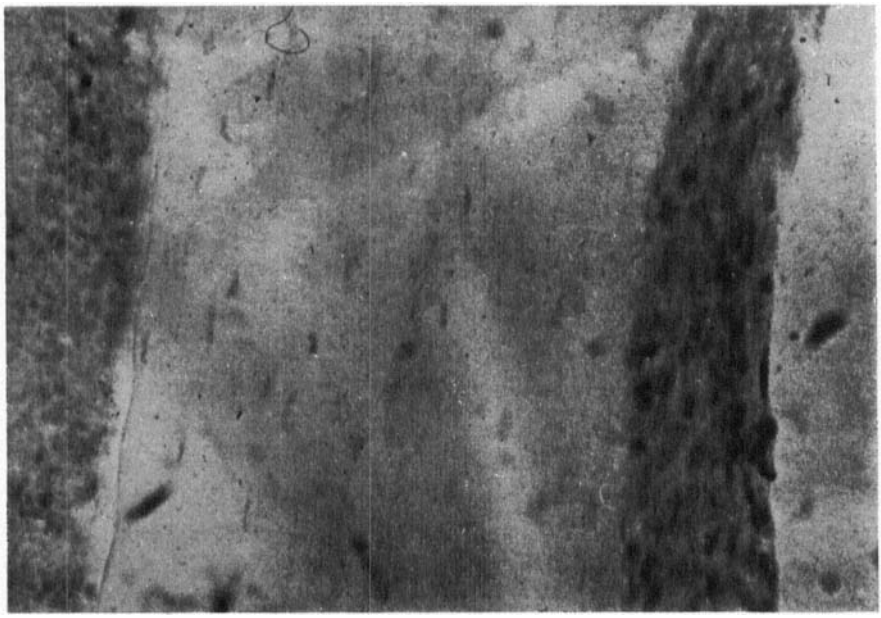

Figure 1 a. Autoradiograph of a fractured tibia from a 5-week-old mouse (× 250).

Figure 1 b. Autoradiograph of the periosteum from a fractured tibia (× 1000).

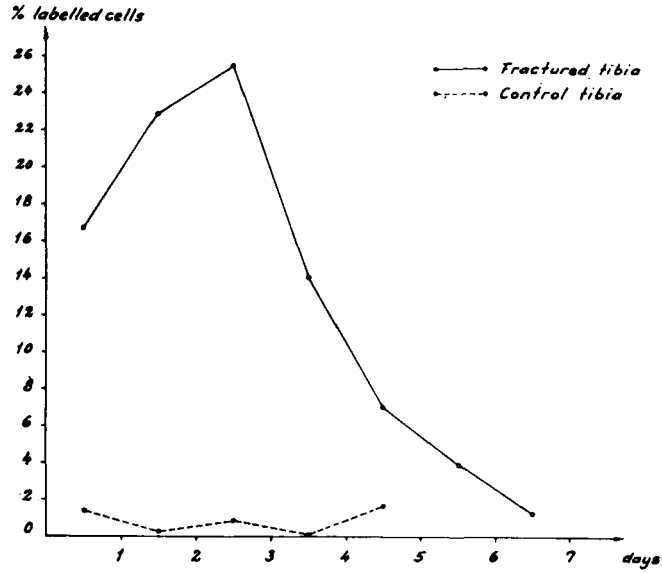

Figure 2. Labelling index in the periosteum from a fractured and non-fractured tibia. Zero time represents the time of the fracture.

one tibia. The contralateral tibia was used as control, supplemented by preparations from two non-traumatized mice.

One hour before the specimens were prepared (after the mice had been killed by an over-dose of ether), tritium-labelled thymidine was injected subcutaneously in a dosage of 1 mC/g. During the first 3 days specimens were prepared every 4 hours from 2 mice. During the subsequent 16 days 2 mice were used each day.

Immediately after sacrifice the bones were dissected free under an operation lens, fixed, decalcified, embedded in paraffin, and cut into 5 μ sections. For the autoradiography we used a Kodak K-5 emulsion. The specimens were exposed for 12 days in dry air at 4° C. After developing and fixation they were stained with haematoxylin-eosin.

Since an additional purpose of this study was to assess the validity of the method as an indicator analysis in studying the union of fractures, the specimens were coded and examined microscopially by two persons independently of each other. A third person adjusted the specimens to a periosteal area near the fracture and a periosteal area away from the fracture, but on the fractured bone, and a diaphyseal as well as metaphyseal area on the control tibia.

A total of 264 counts were made, each representing one field of the microscope at a magnification of $\times$ 1000. No regard was paid to the differentiation of the periosteal cells, the counts merely including labelled and non-labelled cells in the periosteal layer.

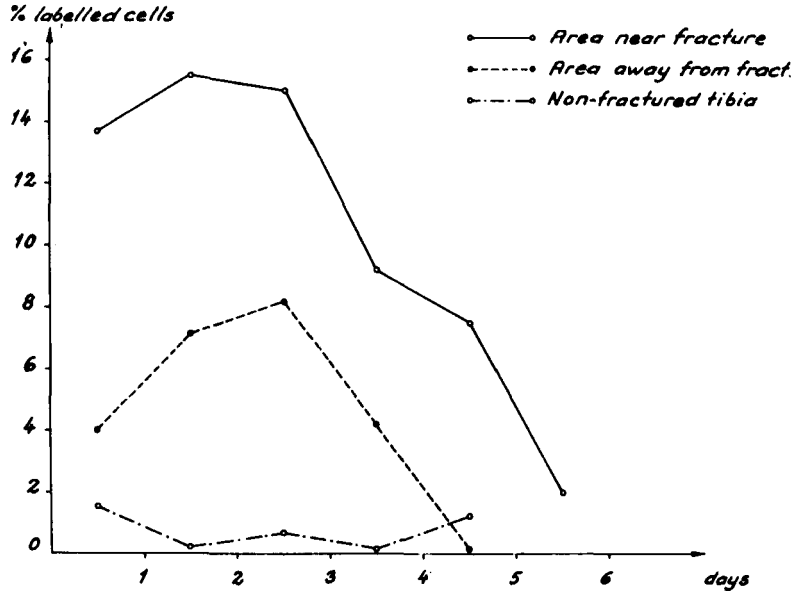

Figure 3. Labelling index in the periosteum from an area near the fracture, an area away from the fracture, and from a non-fractured tibia. Zero time represents the time of the fracture.

RESULTS

Figure 2 is a graphic presentation of the number of labelled cells in the periosteum on the fractured tibia and the control tibia. The values are expressed as per cent labelled, out of the total number of cells counted. The values per 24 hours represent the mean values of counts made during the 24 hours.

There was a significant difference between the number of labelled cells on the fractured bone and on the control bone during the first 3 days (1st day T value 3.323 $P < 0.005$; 2nd day T value 7.530 $P < 0.001$; 3rd day T value 3.315 $P < 0.02$). The percentage of labelled cells on the fractured bone was 16–26, with a distinct peak between the first and the second day. On the control bone the number of labelled cells ranged from 0–2 per cent. After the 7th day the number of labelled cells was the same on both sides.

In Figure 3 the counting results on the fractured tibia are specified for the area near the fracture and the area away from the fracture and compared with the control bone. Here too the values represent the mean of counts during 24 hours. It is apparent that the number of

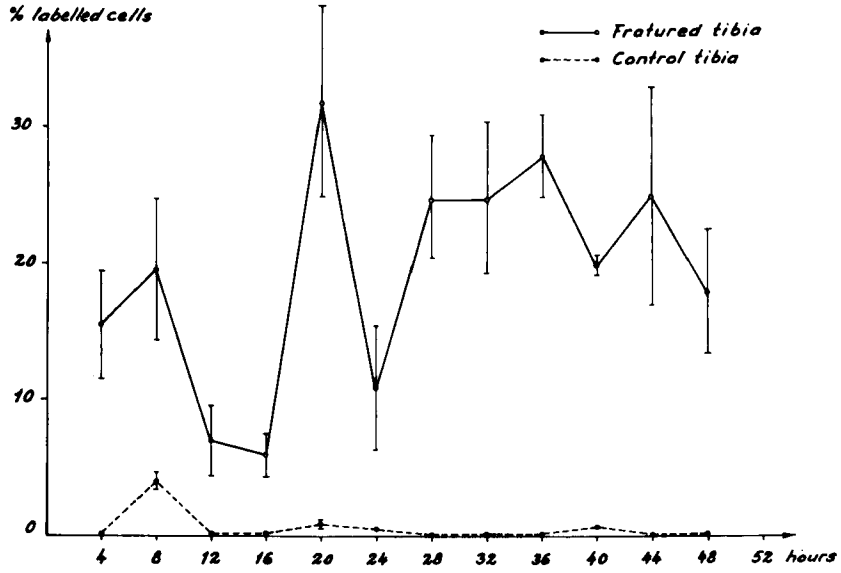

Figure 4. Labelling index showing the standard deviation on the individual counts during the first 48 hours after the fracture.

labelled cells on the fractured bone—near the fracture as well as away from the fracture—ran a parallel course graphically.

On the other hand, there was a significant difference in the labelling percentage between the two areas on the 1st and 2nd day (T value 2.753 $P < 0.02$; T value 2.357 $P < 0.05$), whereas the difference from the 3rd day onwards was not significant.

Figure 4 shows the percentage of labelled cells every four hours on the fractured bone and control bone, stating the standard deviation on the means. From Table 1 as well as from Figure 4 it is apparent that the standard deviation on the individual mean values representing one set of specimens is high (coefficient of variation 14–23 per cent). Moreover, there seems to be a marked variation in the number of labelled cells from one set of specimens to another. On the other hand, there is satisfactory agreement between the two independent countings, the mean difference being 0.08 plus 1.3 with a standard deviation of 4.5 (cf. Table 1).

CONCLUSION AND DISCUSSION

In the present study we found autoradiographic methods to be well-suited to indicator analysis for assessing fracture-induced cellular

Table 1.

Hours after fracture	Number of labelled cells		differ. x–y
	x-examin.	y-examin.	
4	26	27	—1
8	20	20	0
12	12	7	+5
16	6	7	—1
20	40	43	—3
24	21	13	+8
28	84	93	—9
32	47	43	+4
36	46	45	+1
40	29	34	—5
44	12	10	+2
48	18	20	—2
Mean	30	31	0.08
S.D.			4.5
S.E.D.			1.3
Paired "T" = 0.063 ~ P = 0.96 (N = 12)			

proliferation in the periosteum. By the same method Tonna & Cronkite (1961) found cellular proliferation 8–16 hours after fracturing the femora in mice young. The proliferation was at a maximum after 32 hours.

In our study there was a significant increase in labelled cells as early 4 hours after the fracture, reaching a maximum after 20 hours in the area near the fracture. On the other hand, a later response seemed to occur in the area away from the fracture, where the number of labelled cells did not reach a maximum until 28–32 hours had elapsed.

On the contralateral control bone there was a slight increase initially in labelled cells, but thereafter the number was constant and low. The increase did not coincide with the response in the fractured bone. However, as compared with the periosteum on the tibiae of two non-traumatized mice, the periosteal layer was thicker and more cellular.

In other words, the results indicate that a fracture initiates a factor which acts as an inductor upon the osteogenic cells, at first locally, but the reaction at a distance from the fracture as well as the mild response in the contralateral bone might indicate that a universally active factor is operative as well.

SUMMARY

With the aid of autoradiography fracture-induced cellular proliferation in the tibial periosteum was assessed in 5-week-old albino mice during the period one hour to 17 days after the trauma.

The proliferative response set in 8–16 hours after the fracture, reaching a maximum in 20 to 28 hours. The response was most marked at the site of the fracture, but was demonstrable also on the tibial diaphysis and on the contralateral, non-fractured tibia.

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