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PRESENCE OF MICROSCOPIC CRACKS IN VIVO IN BONE

HAROLD L. FROST, M.D.*

Some years ago while the writer was demonstrating the peculiar localization of cracks in ground sections to Dr. Charles O. Bechtol, the latter raised the intriguing possibility that the cracks need not necessarily be all artifact. This and Dr. Bechtol's engineering background provided the stimulus for the investigative work leading to this paper, which is the first of three dealing with the subject.

In the engineer's vocabulary the word fatigue has a special meaning: weakening of a structural member under repeated loads leading to failure. The failure begins as a series of minute cracks which gradually extend in depth and length until the remaining intact material is insufficient to withstand a single load. The study of fatigue is complicated by many factors, among them being surface defects of form and composition, corrosion, the loading, the incompleteness of stress reversal during the loading cycle, and the composition, homogeneity, grain and phase structure of the structural material. The fatigue process is still not well understood, even in extensively studied metals^{2,6}

The definition of fatigue suggests to an orthopaedist that a similar factor might be at work in the human skeleton. If so, one of its manifestations should be the existence of microscopic cracks in the bones. The problem then resolves itself into a two-fold one: unequivocal demonstration of such cracks as an *in vivo* phenomenon, followed by study of the cracks (if found and if we can be sure they are not artifact) in sufficient numbers of patients, pathological states, bones and experimental situations to assign useful meaning to them for academic and clinical purposes.

Unequivocal demonstration of *in vivo* cracks appears to be a simple project but did not prove so simple in execution. First it was necessary to stain such cracks, and second to visualize them on a cross section of bone in such a manner that artifacts could either be prevented or be readily distinguished from *in vivo* cracks. The writer believes that *in vivo* cracks have been demonstrated conclusively and this paper will present the evidence for this belief, leaving to subsequent publication the correlation of the cracks with stress, age, disease and some of the peculiarities of bone mineral elaborated in his laboratory.

MATERIALS AND METHODS

The *Materials* consist of 14 ribs from 14 patients, 10 males and 4 females. The ribs were resected during a thoracotomy in 10 cases and removed at postmortem in 4 cases. The ages of the patients ranged from 18 to 82 years. Because of the possibility that the periosteal elevator used in surgery might produce cracks during periosteal stripping the ribs removed at postmortem were resected with the scalpel.

The *Methods* finally adopted are as follows:

(a) A length of rib 3" or more was immersed in 1% basic fuchsin in a 40% ethyl alcohol solution and allowed to stain therein for 4 weeks. Nearly complete staining of all the physiologic spaces and cracks in the bone results. Staining is done at room temperature and the volume of the solution should exceed the volume of

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the specimen by a factor of ten. Basic fuchsin is so poorly soluble in pure water that unalcoholic solutions are very ineffective bone staining reagents and in addition are plagued by a peculiar failure to penetrate the bone's physiologic spaces. Drying in vacuo prior to staining, staining at elevated pressures or temperatures and staining in 95% or absolute alcohol were rejected at the beginning of this project because of the possibility that they might introduce artifacts.

An essential part of this technique is that the bone specimen to be stained be as fresh as possible when immersed in the stain solution. Prior fixation is not necessary since the alcohol in the staining solution produces what fixation is needed. Dehydration or defatting in any solvent prior to staining introduces the possibility of artifact due to differential shrinkage effects.

(b) At the end of the staining period the bone specimen is transferred from the staining solution to a large volume (exceeding the specimen volume by 100 X or more) of tap water and allowed to remain in this solution with one change to fresh solution for at least 48 hours. This step accomplishes several things. First, sections containing as little as 15% alcohol are unmanageably brittle when ground and will therefore fragment, seriously impairing the usefulness of the preparation. Thoroughly hydrated bone on the other hand may be ground extremely thin in complete cross section since it is then not only quite flexible but reasonably tough.

Second, basic fuchsin is poorly soluble in water but readily soluble in alcohol. If section grinding (the next step) is done with partly alcoholated bone some of the fuchsin will diffuse into the lubricating solution used while grinding and then be able to stain cracks which are artifact while grinding is in process. In thoroughly hydrated bone on the other hand only particulate stained debris may be forced into the exposed surface of artifactual cracks but these cracks will not be stained through the depth of the section. As a result with microscope objectives having numerical apertures exceeding 0.65, cracks stained through the depth of the section can be readily distinguished from those which merely have some particulate matter wedged into the surface but no stain in depth. Such artifacts occur frequently, especially in bone from older patients.

(c) After hydration, the bone specimen is then supported by hand in a wedge-shaped holder fashioned by placing 10 or more folded paper towels in the partly open jaws of a vise. Cross sections about 2 mm thick are sawed from the middle 1/3 of the specimen with a very fine-toothed saw using little pressure and rapid motion. The purpose is to cause as many teeth to perform the cutting action per second as possible. Failure to achieve this may cause the section to fragment during grinding. The section is then ground to a thickness of 30-50 μ and mounted by techniques published elsewhere.³ The grinding should be done under gently running water so that debris is flushed away. Otherwise the section surfaces become covered with adherent debris which interferes with microscopic observation of the section.

(d) From one to 5 complete cross sections per case were prepared and examined. In 10 of the cases longitudinal sections were also prepared to determine the length of the cracks and aid in their localization in the bone. In two of the cases fortuitous

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preparation provided what in essence are serial sections, allowing for the thickness of the bone removed by grinding. Sections which were incomplete due to fragmentation in grinding were not included because of the possibility that the number of cracks might vary systematically in varying quadrants. This proved to be the case.

(e) To be accepted as a crack, the following criteria proved necessary in the light of experience.

First — the crack had to be stained through the depth of the section. Any crack not so stained was considered artifact in spite of the probability that some of them were *in vivo* cracks unstained due to poor penetration of stain.

Second — the crack had to open visibly onto the surface of the section. The air drying preceding mounting in resin usually (by virtue of a small amount of shrinkage occurring during drying plus variations in lamellar orientation and degrees of mineralization in different parts of the section) causes cracks opening into the section surface to spread apart slightly so that optically resolvable space exists in the crack.

Third — There had to be no permeation of the stain into the bony substance in the walls of the crack. Repeatedly the writer has observed, during the examination of routinely prepared ground sections, a zone of fuchsin permeability spreading outward from the surfaces of cracks caused both accidentally and deliberately (while evaluating the effect of various methods of section preparation on the permeability of fresh bone). This zone of fuchsin permeability varies from several to 50 μ or more and for the present purpose must serve as indication of probable artifact.

(f) Quantitative determination of *in vivo* cracks is not attempted in this paper. Before we walk we must learn where to walk. Such determination would necessitate the following conditions and measurements: knowledge that every *in vivo* crack present can be stained; accurate counts of the number of cracks, total length and average length of cracks per unit area of cross section; homogeneity — or lack of it — of cracks per unit area at various levels in various bones plus correlations with cortical thickness, magnitude of physical stress and associated inhomogeneities of bone such as vascular porosity, feathering, micropetrosis, osteocyte death and proportions of Haversian to extrahaversian bone. It becomes evident that these are matters for future work and technical development.

(g) While observing the cracks which were present and which fulfilled the criteria listed under (e) it became evident that there were at least 4 types according to distribution. These types are cracks occurring in the plane of cement lines, cracks running parallel to lamellae and cracks crossing lamellae obliquely or perpendicularly. A much smaller group consists of cracks which invade Haversian systems. The counts of cracks are so tabulated in Chart I.

RESULTS

The information in Charts I and II will be summarized here, and in addition some interesting features of tentative significance will be described which are not presented in the Charts.

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CASE	AGE	SEX	OR PM	COMMENT	SECTIONS EXAMINED	TOTAL NUMBER OF CRACKS SEEN			
						Parallel Lamellae	Perpendicular Lamellae	In Cement Line	In Haversian System
924416	18	M	OR	Dyspnea	2	1	1	23	1
924957	21	M	OR	Hiatus Hernia	3	1	2	3	0
914892	22	F	OR	Bronchiectasis	2	0	1	1	0
927890	23	F	OR	Patent Ductus	3	1	1	15	0
933423	26	M	PM	Acute Trauma	3	1	2	1	0
831088	28	M	OR	Hematemesis	2	0	11	5	0
459576	48	M	OR	Cough	5	16	39	33	1
856829	55	F	OR	Osteomalacia	3	4	3	7	0
928071	55	M	PM	Dyspnea	2	11	3	18	0
926196	57	M	OR	Asymptomatic	2	3	14	11	0
722345	58	F	OR	Hiatus Hernia	3	0	1	6	0
929122	58	M	OR	Ca. Esoph.	2	6	6	13	0
135720	61	M	PM	Cough	3	2	8	3	0
015364	82	M	PM	Myo-Infarct.	1	2	0	1	0

Chart I

Number and Placement of Cracks Seen

OR=Specimen from operating room

PM=Specimen obtained post mortem

CASE	CRACKS PER SECTION		
	Parallel	Perpendicular	Cement
924416	0.5	0.5	11.5
924957	0.3	0.6	1.0
914892	0.0	0.5	0.5
927890	0.3	0.3	5.0
933423	0.3	0.6	0.3
831088	0.0	5.5	2.5
459576	3.2	7.8	6.6
856829	1.3	1.0	2.3
928071	5.5	1.5	9.0
926196	1.5	7.0	5.5
722345	0.0	0.3	2.0
929122	3.0	3.0	6.5
135720	0.6	2.7	1.0
015364	2.0	0.0	1.0
Average for Group	1.1	2.2	3.9
Average for Group less than 30 years old	0.23	1.3	3.5
Average for Group more than 30 years old	1.7	2.9	4.2
12 Males	1.7	2.9	4.5
4 Females	0.4	0.5	2.4

Chart II

Number of Cracks per Section

(A) In all sections examined some cracks fulfilling the criteria in Methods, (e), were found. 36 complete cross sections were examined.

(B) The localization of the cracks in extrahaversian bone* is striking. Of a total of 280 acceptable cracks examined only 2 invaded Haversian systems, the remaining 278 ending rather abruptly at the cement line separating Haversian systems from extrahaversian bone.

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(C) 50% of the acceptable cracks examined occurred in the plane of cement lines, while 33% were oriented in the cross section perpendicular to lamellae and 17% ran parallel to the lamellae. 1.8% crossed cement lines to continue in adjacent lamellae and only 0.7% entered Haversian bone as defined in footnote (*). (Slide rule accuracy only).



Figure 1A

50X, cross section 28 year man's rib stained and sectioned as described.

(D) Many planes, particularly in cement lines, were seen which stained in depth with fuchsin but which did not have a visible physical crack. These stained planes in cement lines occur within a micron of the outermost, optically distinguishable edge of the cement lines and are themselves only a micron or so thick. Another, less frequently observed phenomenon was the appearance in extrahaversian bone of somewhat similar plans of fuchsin staining which were quite wavy and fibrillated in

*Footnote: Extrahaversian bone is for the purpose of this paper defined as all bone in a section which is not intact Haversian systems. In so-called double and triple systems, only the inner system or ring is considered Haversian, the remainder being included in the extrahaversian moiety. Fragments of remodelled Haversian systems are also considered to be extrahaversian. This classification is not entirely arbitrary and is based on other work from the writer's laboratory wherein consistent differences in brittleness, hardness, osteocyte death, micropetrosis, feathering, permeability, susceptibility to etching in acid buffers and in shrinkage exist between the Haversian and extrahaversian moiety as defined herein. Previous definitions of extrahaversian bone (interstitial lamellae) lack sufficient precision for the present needs.

appearance but which also could not be shown to possess the physical discontinuity needed to define them as cracks. It is suspected that these are cracks in genesis. (Figure 4).

(E) In some sections careful examination would reveal cracks which, when followed down into the depths of the section, gradually or suddenly expanded, the space occurring having stained cells on the surfaces of the walls and in some instances a capillary vessel in the lumen. The walls of such spaces are often partly scalloped by Howships lacunae. (Figure 3),

(F) Attempts to correlate resorption spaces with the presence of cracks have so far failed to show a correlation, although such a correlation is anticipated by the writer.

(G) In cases 459576 and 831088 the final sections were ground from portions of the ribs sufficiently adjacent that continuity of trabecular and Haversian structures is evident when comparing the sections. In these sections the cracks which can be seen on one of them are found to continue in the same direction and moities of the extrahaversian bone in the section from the adjacent level. Such continuity is especially convincing when one has doubted, as the writer has, the conclusiveness of this findings. (Figures 1 and 2).



Figure 1B

Same as 1A, but higher power to reveal cracks (outlined running longitudinally in a moiety of extrahaversian bone.

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Figure 2A

Same specimen, 50X, section less than 1.0 mm distant from section in Fig. 1. The trabeculum at right leads to a moiety of bone which is continuous with the moiety comprising the lower portion of the trabeculum seen in Fig. 1A.

(H) Examination of longitudinal sections prepared from the present material reveals that the cracks course in length to a variable degree ranging from 20 - 200 μ and occasionally longer. On cross sections the cracks range in length from 10 - 100 μ , occasional longer ones being seen in cement line planes between different portions of circumferential lamellae.

(I) The limited amount of available material suggests that cement line cracks occur with greater frequency in bones from young patients than in bones of older patients. Older patients on the other hand seem to develop more cracks in the extra-haversian bone than young ones.

(J) There does not appear to be any significant difference in the incidence of cracks between material obtained from the operating room and that removed at postmortem.

(K) Artifactual cracks are found in most, but not all, of the 42 cross sections examined. By virtue of poor stain penetration some of them are probably *in vivo* cracks but cannot be included as such in the present work. Even in completely stained sections artifacts are usually evident and are unstained. Occasionally the

stresses produced during grinding lead to the extension of an *in vivo* crack. These are recognized by the stained *in vivo* portion and unstained artifactual portion.

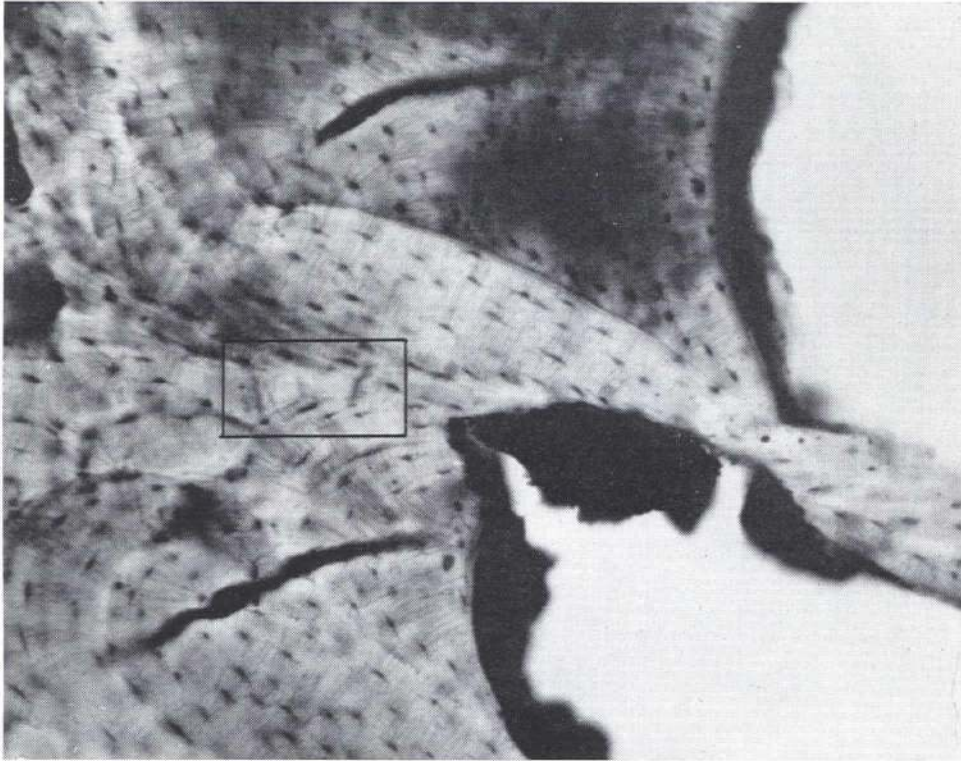


Figure 2B

Higher power of 2A. Three cracks are contained in the rectangle.

DISCUSSION

It is a moot point at present whether the cracks demonstrated by the writer are fatigue cracks or not. This must be proved by further work. The inference that they are the result of a fatigue mechanism is clear however. It will be of considerable interest to see if, as many suspect, these cracks play an important role in the production of some hip fractures, some spontaneous vertebral collapses in osteoporosis, the genesis of some cases of spondylolisthesis and the migration of some endoappliances attached to or partly embedded in bone. The writer has partial data available which suggest that this is the case.

If cyclic loading of living bone is the cause of the cracks, rather than some other less obvious factor, then the distribution of the cracks suggests that the affected parts of the bone are either weaker or subjected to larger stresses or both and it will be of interest to investigate and quantitate these factors.

The pronounced tendency of Haversian systems to be avoided by *in vivo* cracks and as previously reported by grinding cracks^{4,5} leads one to the conclusion that they are either much stronger than extrahaversian bone or much more elastic. The first thesis cannot be supported in the light of available evidence. In addition to work from this

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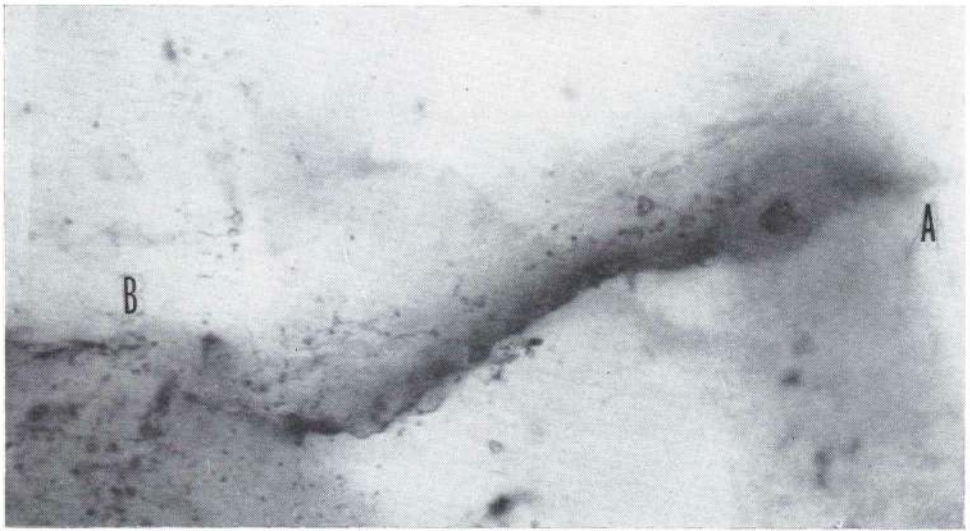


Figure 3A

Cross section 48 year man's rib. Focused at surface of section revealing serpentine winding of crack from the endosteal surface (Point A) to the edge of an osteon (at point B).

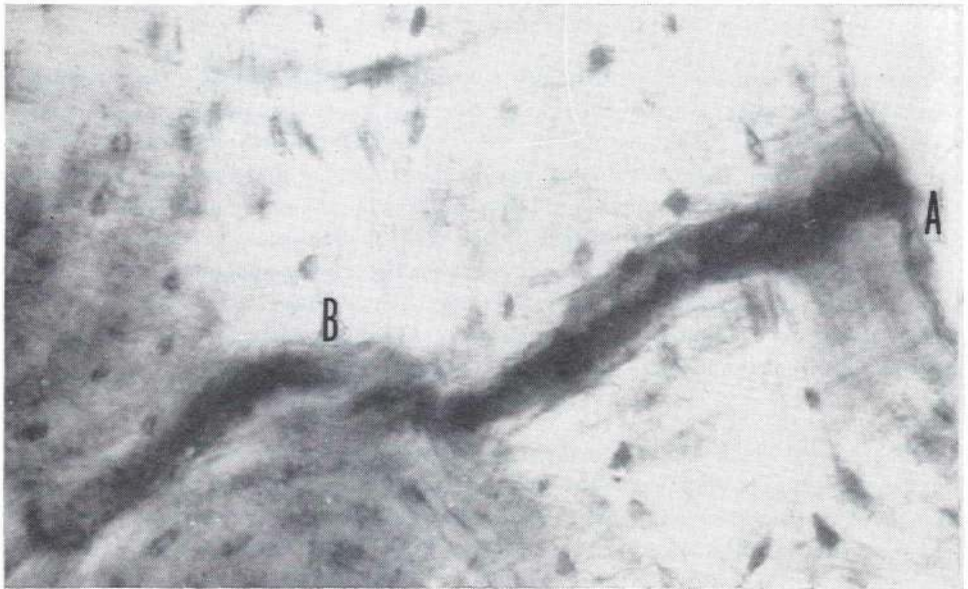


Figure 3B

Same field as 3A but less magnification, plus Wratten 25 filter. A vascular channel underlies the crack and contains a capillary vessel. Focused 45 μ under section surface.



Figure 4

Another field from same section as Fig. 3, the line labelled A is a crack stained in depth through the section. The line labelled B is a plane of Fuchsin staining which is not yet a crack. Both planes lie in the cement line separating the bone above from that below.

laboratory which indicates Haversian bone, particularly in older people, to be less densely mineralized than extrahaversian bone, most published microradiographic studies demonstrate the same thing. It is also known with reasonable certainty that the amount of matrix, once formed, does not change during mineralization and that the initial water content of the matrix is high but diminishes as mineralization density increases, the lowest water content (exclusive of that lying in bone's physiologic spaces) occurring where the greatest degree of mineralization exists. It is known that poorly mineralized bone in bulk is more easily strained than normally mineralized bone.¹ These facts, when assembled, make it awkward to assume that the sparing of Haversian systems from cracks is on any other basis than an increase in flexibility compared to the surrounding extrahaversian bone.

The cracks which lead down to a space containing some cells on the walls and occasionally a capillary are suggestive of a repair process. Certainly if the *in vivo* cracks occur by a fatigue mechanism they must either be repaired or accumulate, leading in the latter event to collapse of the entire skeleton. With certain reservations we know this does not occur. Therefore, the cracks cannot be accepted as fatigue in nature unless a repair process which removes them is also observed. While the writer feels that the spaces observed and described with their occasional Howship's lacunae probably are crack repair processes, it is necessary to observe the end product and the transition

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phases of the phenomenon before feeling can be substantiated. These observations have not yet been made.

The nature of the strains and other factors causing the cracks is of course speculative. However, their longitudinal orientation is consistent with the thesis that shearing strains are the predominant cause. The evidence supporting this thesis is: the known production of horizontal shearing strains under bending which are maximal in the plane of the neutral axis; the known production of longitudinal shearing strain in a structural member under torque;⁶ the known weakness of cortical bone under longitudinal shearing strain as opposed to radial or tangential shearing strain;¹ and the known fact that torque and bending loads are the most frequent loads our long bones are subjected to.

SUMMARY

Three-inch or longer lengths of fresh, intact ribs obtained from the operating room and at postmortem were stained, sectioned and examined for *in vivo* microscopic cracks, utilizing simple techniques and procedures. Cracks which the writer interprets as existing *in vivo* were observed and exhibit a curious predilection for cement line planes and extrahaversian bone.

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