SAMPLENG BONES FOR MICROCRACKS

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INTRODUCTION
Fatigue microdamage is a normal feature of bone. Of particular interest are microcracks (μcks) ~80 μm long in histologic sections stained with basic fuchsin. While μcks apparently play an important role in bone mechanobiology [1], many microscope fields must usually be examined to find a single microcrack. Consequently, the experimental problem is one of tedious, time-consuming measurements, and there is a tendency to use marginally adequate sample sizes (histologic sections) that risk Type II errors in the sense that no cracks are found in the specimens from some individuals. However, "crackless samples" suggest that the sample size was simply too small to measure crack density (Cr.Dn) with the desired sensitivity. This paper presents a statistical model to help choose a suitable sample size a priori that will reduce the probability of crackless samples in a predictable manner.

METHODS
To the degree that μcks are rare in individual microscope fields, and also randomly distributed among these fields, the probability that an arbitrarily chosen field will contain k μcks can be approximated by the Poisson distribution:

\[ P(k) = e^{-\mu} \frac{\mu^k}{k!} \]  
(1)

where \( \mu < 1 \) is the mean number of cracks/field and k is the number of crack-containing fields existing in the sample. We therefore adopted this statistical distribution as our model.

Assuming the sample size (\( A_s \), mm² of section) needed to avoid crackless specimens is a surrogate criterion for minimally adequate sampling, it was shown that

\[ A_s = \ln \left[ \frac{1}{1-(1-P^*)} \right] / \text{Cr.Dn} \]  
(2)

where \( P^* = 1 - \exp(-\mu) \) is the probability that a sample will contain one or more μcks. Conversely, since \( \mu = A_s \times \text{Cr.Dn} \), Eq. 1 implies that probability of a crackless specimen is

\[ P(0) = \exp(-A_s \times \text{Cr.Dn}) \]  
(3)

Using a data analysis computer program (Statview), 10 columns of random numbers were generated, each having a Poisson distribution, a theoretical mean of 0.10 (cracks/mm²), and simulating μcks counts in N = 5000 microscope fields from an imaginary bone. This process was repeated 54 times, with N decreasing in an arbitrary manner to simulate reduced sampling of the 10 specimens. Then the procedure was repeated for a theoretical Cr.Dn of 0.01 mm².

Model results were compared to 8 sets of cortical bone data, each involving multiple specimens: A) canine ribs treated with a placebo or one of 2 bisphosphonates [2]; B) human femur specimens [3]; and C) human radius and ulna specimens stained en bloc with 2 different methods [4]. Thus, individual bones had been treated in various ways likely to have increased Cr.Dn variability. These 8 data sets enabled comparisons between model predictions and actual values of percent crackless specimens.

RESULTS
Fig. 1 shows the effect of N, on the coefficient of variation (CV) of the resulting Cr.Dn sample values. The relationship is hyperbolic and for a nominal crack density of 0.10 mm² the CV rises rapidly as N, falls below 100 fields. When the nominal μck density is only 0.01 mm², substantially more fields must be sampled to obtain a similar CV. The occurrence of crackless samples is also related to the CV, and did not begin in either model until the CV surpassed ~0.5 (inset). Thereafter, the % crackless specimens increased in proportion to CV, but with considerable variability. Regression of the actual numbers of crackless specimens in the 8 data sets against the predictions of Eq. 3 gave \( y = 4.32 + 0.911x \), with \( R^2 = 0.946 \) and \( p < 0.0001 \), demonstrating the usefulness of the present model (Fig. 2).

DISCUSSION
Fig. 1 shows that the probability of finding no cracks in a field depends on the CV, which in turn depends on the number of fields sampled/specimen. A high CV is symptomatic of insufficient sample size. However, in general, the probability of a crackless sample depends on both Cr.Dn and sample size. Therefore, the result shown in Fig. 1 is a specific example of a general rule that crackless samples result from insufficient sampling.

Similarly, Eq. 2 shows how the sample size needed to avoid crackless samples depends directly on the fraction of microscope fields that contain cracks. Because this fraction does not depend specifically on the assumption of a Poisson distribution, the present results are a special case of a general rule that appropriate A, is determined by the fraction of crackless fields. However, Eq. 3 is a result that depends on Poisson statistics and was used to calculate Fig. 2. Therefore, the high R² of Fig. 2 supports the assumption that the probability of μcks in microscope fields is well approximated by a Poisson distribution.

Finally, these results suggest caution in the interpretation of experimental results involving samples containing "crackless specimens."

Fig. 1: Dependence of coefficient of variation of Cr.Dn and sample size. Mean values = 0.10 and 0.01 mm² for filled and open symbols, respectively. Inset shows crackless samples increase substantially as coefficient of variation increases.

Fig. 2: Actual vs. predicted values of percent crackless specimens for the 8 sets of cortical bone data.

ACKNOWLEDGEMENT
Supported by NIH grant AR41644.

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51st Annual Meeting of the Orthopaedic Research Society
Poster No: 1540