microscopic and chemical data, but we submit that both of these present challenges fail.

The structure illustrated in our earlier paper[3], and reproduced by Bailey *et al.*[1], is, as we pointed out, one of several possible quasi-hexagonal packing schemes, all with the same unit cell and consistent with the X-ray diffraction data[4,5]. One of these schemes[5], already preferred in cross-linking arguments, is able to incorporate the 1,5,1,5 cross-links[1,6] to a greater extent than any other hexagonal model including the 'compressed microfibril' model[7]. The paper by Miller and Tochetti[5] was in the press and thus not available to Bailey *et al.*[1] at their time of writing and the cross-links used by the former were not those to which Bailey *et al.* drew attention. Contrary to the conclusions of the latter, we have shown that the cross-linking data are consistent with quasi-hexagonal packing, and support the particular packing scheme mentioned above.

The characteristic 3.8-nm row-line in the X-ray pattern from native tendons is often replaced by a row-line in the range 4.5–18 nm in X-ray patterns from fixed or stained specimens[8–10]. It is possible that the arguments of Squire and Freundlich[2] are based on such an artefact. It is difficult to preserve the 3.8-nm spacing in specimens prepared for electron microscopy but this has been achieved[11] by monitoring the effects of the preparative steps using X-ray diffraction. Furthermore, the observation of Parry and Craig[12] might be due to 4-nm units adhering to the circumference of the fibrils. Finally, Bailey *et al.*[1] refer to a published X-ray pattern[10] of stained tendon showing an 8-nm row-line as strong evidence for an 8-nm lateral unit cell. This is invalid in view of the variability described here.

Hence, while fully accepting that X-ray, electron microscopic and cross-linking data are all relevant, we do not believe that other studies[1,2] improve on the simple quasi-hexagonal model.

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SQUIRE AND FREUNDLICH REPLY—Hulmes and Miller have suggested that our observation of a lateral periodicity in electron micrographs of collagen fibrils need not be taken into account in considering possible collagen packing schemes as we are probably studying preparative artefacts. They also imply that the variation of lateral periodicity with different preparative treatments is significant. The following comments may help to put these views into perspective.

(1) Published electron micrographs have frequently shown collagen fibrils which seem to be composed of longitudinal strands[1,2]. Previous studies of such preparations (for example, by optical diffraction) have failed to demonstrate any regularity between these strands, thus they could have been taken to be artefacts of the preparative procedure in which fibrils which are close-packed *in vivo* are split randomly by the effects of fixation, staining and drying. We have shown using the convolution technique[6], that such preparations can be very much more regular than has previously been thought. Of course we do not know that collagen molecules occur in such spatially separate bundles *in vivo*; electron microscopy is *per se* a study of preparative artefacts. We would argue, however, that even if molecular clumping in collagen fibrils occurs as a result of the preparative procedure, periodic clumping, however caused, is most likely to be a reflection of a periodic variation in the intermolecular interactions in the native fibril. Thus at the very least our results imply a variation every 50–100 Å in the intermolecular interactions in collagen fibrils. Note that three different preparations gave rise to periodicities in the limited range 50–100 Å.

(2) In the light of the observed variation in the D spacings in our fibril preparations, even within a single fibril, the observed range of values of the lateral spacing was by no means surprising. When analysing our data we considered the postulate[3] that collagen fibrils are composed of 80 Å structural units. Despite the observed range of spacings our data are clearly consistent with this postulate. However, the data do not prove this, thus we were careful to note that they are also consistent with a superlattice structure viewed in different directions.

(3) The quasi-hexagonal packing scheme of Hulmes and Miller[4], which is a very attractive model, is essentially an array of equivalent molecules each ~15 Å in diameter and each making identical interactions with its neighbours. There is no reason *a priori* why in such an array there should be a regular variation in interactions every 50 to 100 Å, nor is there any reason in the quasi-hexagonal model why collagen fibrils should be built up from 40-Å units. Hulmes and Miller suggest this to explain the results of Parry and Criag[3] but, although the quasi-hexagonal model has a unit cell with a principal interplanar spacing of 37.8 Å, this is not a structural unit. The largest structural unit in the model, as proposed[4], is an individual collagen molecule 15 Å in diameter.

(4) We consider it likely that, superimposed on the basic quasi-hexagonal lattice, there is a pattern of intermolecular interactions, including specific cross-links, which defines molecular groupings on a larger scale (that is 50–100 Å). The models of Trus and Piez[5] represent examples of this kind of structure; indeed Hulmes and Miller[4] themselves acknowledge such a possibility. We hope that our studies of cryo-sectioned collagen fibrils[6] will help to define the size and nature of these larger molecular groupings for which X-ray diffraction has so far failed to provide tangible data.

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