Although Dr Muir and Dr Hardingham have been able to confirm this in pig cartilage, they found that not all proteoglycans are capable of aggregation when labelled and unlabelled molecules are combined. In pulse-chase experiments *in vitro* during 6 hours, no precursor-product relationship between the low and high molecular weight forms of proteoglycan was evident and each form was labelled to a different degree independently. This result is not consistent with the idea of a single subunit, but rather with a heterogeneous population of proteoglycans, synthesized *de novo*, which provides cartilage with a range of physical properties for optimal function.

Professor D. S. Jackson (University of Manchester) considered the degradation of insoluble collagen and summarized the current hypotheses, namely the lysosomal enzyme and the neutral collagenase theories. Recent studies have indicated that the neutral collagenase has no effect on insoluble collagen and Professor Jackson reported that in his laboratory the neutral collagenase from synovial fluid had no effect on polymeric collagen. In contrast, the lysosomal extracts have long been known to possess enzymes which are capable of degrading collagen, and Professor Jackson confirmed these studies, demonstrating the fragmentation of the fibre by electron microscopy. Because the neutral collagenases are capable of cleaving the tropocollagen molecule, Professor Jackson suggests that the degradation of collagen *in vivo* is achieved by fragmentation of the fibre by the lysosomal enzymes, followed by cleavage of the tropocollagen molecules with the neutral collagenase.

The meeting concluded with a short talk by Professor E. Kulonen (University of Turku) on the evolution of collagen. Professor Kulonen and Dr J. Pikkarainen have carried out an exhaustive analysis of the amino-acid composition of collagen from a wide range of species. During evolution there seems to be a tendency towards the development of more stable forms, indicated by the increasing ratio of the amino-acids to serine + threonine. The $\alpha 1$ chain is more stable than the $\alpha 2$ chain and this is reflected in its higher denaturation temperature. Professor Kulonen suggests that this divergent development indicates that a triple helix composed of similar chains existed in the ancestral collagens. Support for this hypothesis has been obtained by electrophoresis; the invertebrate collagens seem to have a single $\alpha$ chain whereas the higher vertebrates have two or three distinguishable $\alpha$ components. What benefit the organism derives from differentiation of these three chains remains to be elucidated.

# CORRESPONDENCE

## Role of DNA Polymerase

SIR,—Following the isolation of *Escherichia coli* mutants lacking demonstrable levels of DNA polymerase I, it has become widely accepted that this enzyme has no role in replicative DNA synthesis. The editorial comments of this journal, in particular, have repeatedly emphasized this conclusion, despite the modesty of the claims by those who first produced *pol* $A^-$ strains. We would like to point out certain lines of reasoning which suggest that polymerase I may be a valid model for the study of replicative DNA synthesis in *E. coli*, and perhaps plays a direct role in it. Some of the points raised may already have been answered by research of which we are unaware. Nevertheless, we think that their cumulative weight should temper the haste to exclude polymerase I (and now polymerase II) from any role in replication.

First, we recall the well-known fact that no template-dependent DNA polymerase has been isolated which does not resemble polymerase I in its preference for "repair" synthesis *in vitro*. This is true of both eukaryotes and prokaryotes, to the best of our knowledge.

Secondly, in a case where strong evidence links a DNA polymerase with replication[1], the gene 43 product of bacteriophage T4, the polymerase again acts like polymerase I *in vitro*, requiring both a template and a primer, and differs from polymerase I primarily in its apparent lack of a 5′ exonuclease activity.

Thirdly, it has not yet been shown convincingly that the *pol* $A^-$ amber mutants themselves do not have polymerase I activities. We recall the difficulty of initial attempts to demonstrate polymerase activity in extracts of these cells, from which polymerase II has now been isolated. We feel that such measurements are both difficult and, where negative, questionable. The reason for this particular concern, of course, is that it is clearly established that polymerase I fragments of molecular weight 76,000, and quite likely even lower molecular weights, have polymerase activity. An amber fragment may thus be active in *pol* $A^-$ cells, but not demonstrable *in vitro*. As pointed out in the first report on *pol* $A^-$ mutants, a total deletion of the *pol A* gene would be required to prove that the mutant has none of the *pol A* gene product, and that polymerase I plays no role in replicative synthesis. The fact that such mutants have not yet been discovered is consistent with the opposite conclusion.

Fourth, it remains to be shown that polymerase II is not an amber fragment of polymerase I, although this should soon be known. Its sulphydryl sensitivity, and its insensitivity to antibody to polymerase I, could be caused by a changed conformation, for example. Alternatively, polymerase II may turn out to be a duplication product of the *pol A* gene which has undergone independent mutations. Such gene duplication phenomena of centrally-important functions would be consistent with modern biological concepts. Although polymerase I may, indeed, be expendable for replication, polymerase II could turn out to be a "back-up" system with similar properties. Finally, we should like to point out the ability of polymerase I to "replicate" defined DNAs of the type synthesized in Professor H. G. Khorana's laboratory. In the proper conditions, the enzyme will make as many as 100 copies of a given polymer, and these copies will have the same properties (complete bihelicity, separable complementary strands, high molecular weight) as the template.

A comment on the DNA pieces first described by Okazaki seems inevitable, because many researchers have assigned them a central role in DNA replication. The "Okazaki fragments" could be taken as indirect evidence for the DNA replicase being similar to polymerase I, because they offer a mechanism whereby both strands can be elongated by 5′ to 3′ synthesis. Recent evidence from Kozinski's and Werner's laboratories suggests that Okazaki DNA is not a precursor of high molecular weight, newly-replicated DNA, and may only coincidentally be associated with the growing fork. We feel that evidence dissociating Okazaki fragments from the replication of DNA should not be used to argue against the involvement of polymerase I in this process.

On the basis of these arguments, we hope that some readers will decide that the involvement of polymerase I, or II, in replication is still open to question. At the least, these enzymes should be considered as valid models for *in vitro* studies of replication.

Yours faithfully,

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[1] Speyer, J. F., and Rosenberg, D., *Cold Spring Harbor Symp. Quant. Biol.*, **33**, 345 (1968).

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