Oxygen-Collagen Priority and the Early Metazoa Fossil Record

Kenneth M. Towe

DEPARTMENT OF PALEOBIOLOGY, SMITHSONIAN INSTITUTION, WASHINGTON, D.C.

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Abstract. The thesis is developed that a low oxygen level Precambrian atmosphere presented early-evolving metazoa organisms with physiological connective tissue priorities resulting from the important molecular oxygen requirements in the biosynthesis of collagen hydroxyproline. Shells, cuticles, and carapaces which are not mandatory metazoa prerequisites but which directly or indirectly demand substantial connective tissue collagen are oxygen expensive, low priority features. A marked increase in atmospheric oxygen level near the beginning of the Paleozoic would eliminate oxygen-collagen priorities simultaneously and on a world-wide basis in all metazoa stocks providing evolutionary pressure for enlarged musculatures and associated "hard parts." This could explain the sudden presence in the fossil record of the early Cambrian of advanced and diversified metazoa, the earlier forms of which were essentially unpreservable.

One of the most striking and enigmatic aspects of paleontology has been the sudden appearance of advanced and diversified metazoa organisms in the early Cambrian. This subject has been the object of considerable research and speculation and numerous hypotheses have been proposed to explain the phenomenon. Most of these hypotheses assume that advanced Precambrian Metazoa existed but have not been preserved in the fossil record. This lack of preservation has been attributed to the unavailability of CaCO3, acid oceans, metamorphism, "Lipalian" interval, or the absence of Precambrian coastal sediments. Simpson has evaluated most of these suggestions, and more recently Cloud has summarized and discussed the problem at length. Among Cloud's more important conclusions are the following: (1) "There are as yet no records of unequivocal Metazoa in rocks of undoubted Precambrian age." (2) "The availability or lack of CaCO3 is not the explanation for the distributions of fossils observed." (3) "There is no good reason why we should not expect to find records of Precambrian Metazoa if they were present."

With these conclusions as a foundation, Cloud pursued earlier suggestions and proposed that the "... more or less simultaneous attainment of a metazoa grade of organization by different pre-metazoa stocks... may have been brought on by increase in atmospheric oxygen to levels consistent with metazoa oxidative metabolism near the beginning of Paleozoic time."While this hypothesis may explain the rapid evolutionary diversifications in...
the early Paleozoic, as well as some related inorganic Precambrian events, it still
does not explain the sudden appearance in the early Cambrian of highly orga-
nized metazoans. Regardless of whether the time of diversification was rapid or
not it is still pertinent to ask where the more primitive metazoan-grade ancestors
to these already complex organisms are or were.

The idea that ancestral metazoan stocks existed in the Precambrian but were
not recorded as fossils contradicts one of Cloud's most important and basic
conclusions (3, above). All of the previously published supporting ideas for this
concept have been examined\(^1\) and found wanting. On close inspection, how-
ever, the crux of the argument really rests on the words "no good reason." Clearly,
an explanation is needed that could withstand a reasonable level of
criticism and at the same time be consistent with the bulk of observable data.
Any such explanation, to be acceptable, must allow for early metazoan evolution
while at the same time denying or minimizing the fossil record. On the other
hand the simultaneous and sudden occurrence of diverse preservable forms, inde-
pendent of calcification, must also be satisfactorily explained. Recent advances
in understanding the biochemistry of connective tissue may provide just such
an explanation.

**Distribution of Collagen.** Dense fibrous connective tissues consist princi-
pally of collagen which is a biochemically unique structural protein, generally
of mesodermal origin. It is one of the most abundant animal fibrous proteins
occurring in representatives of every metazoan group, being the dominant extra-
cellular fibrous protein in all metazoan stocks from the most primitive sponges
on up to man.\(^2\) It has even been reported to occur in a protozoan Foraminifera.\(^6\)
Among the vertebrates it occurs widely in both the calcified and uncalcified
states. Among the invertebrates (reports to the contrary notwithstanding) it
occurs only in the uncalcified state serving primarily as a connective tissue.
Collagen connects, supports, and surrounds other tissues and can be considered
the "tape" and "glue" of the metazoan world.

By way of specific examples of the occurrence of collagen the annelid cuticle is
constructed of this protein.\(^7\)\(^8\) The anthozoan body wall is predominantly
collagen, and keratose and other sponge fibers are collagen. The coelenterate
mesoglea is collagenous and in some the float is collagen. The echinoderm body
wall is collagen\(^9\) and echinoderm endoskeletal calcified elements are held together
by collagen.\(^9\) Pelecypod adductor muscles are bound and attached to the shell
with collagen\(^10\) and byssus fibers are collagenous.\(^7\) The brachiopod lophophore
is supported by collagen and the pedicle is collagenous.\(^11\) The complex muscu-
lature and "tendons" of arthropods involve intimate association with collagen.\(^7\)\(^12\)
The gill and endocranial cartilages of the horse-shoe crab *Limulus* contain col-
lagen; the gastropod odontophore is collagenous,\(^12\) as are the subcuticular tissues
of the spiny lobster and blue crab.\(^14\) The examples of collagenous tissues among
the invertebrates are legion and serve to illustrate its structural importance and
widespread occurrence.

It is the principal thesis of this paper that in the absence of, or with the minimal
use of, connective tissue collagen the chances of preservation of ancestral meta-
zoan organisms so poorly endowed would be so low that from a realistic paleontological point of view they would appear nonexistent.

**Structure of Collagen.** Our knowledge of the detailed structure of collagen is still in a state of transition although there is general agreement among most workers that the unit tropocollagen molecule is a triple helix, coiled-coil structure made up of three peptide chains held together by one or two interchain peptide hydrogen bonds per three amino acid residue repeat.\(^{15}\) The structure demands that every third amino acid by glycine. A substantial number of the other amino acids are proline or hydroxyproline. With a few exceptions,\(^{16-19}\) hydroxyproline (and hydroxylysine as well) is often considered unique and characteristic for collagen. This molecule is about 2800 Å long and 14 Å wide and is constructed of about 1000 amino acid residues which are 2.86 Å apart. These basic fibers aggregate to form the larger scale native-type collagen fibrils. The fibrils, when large enough, are characterized by a 600–700 Å periodicity which is observable by both electron microscopy and small angle X-ray diffraction. When aggregated in this macromolecular configuration, the fibril has a high tensile strength which is responsible for its remarkable properties in animal connective tissues. Most workers agree that for any protein to be classified as collagen it must have a wide angle X-ray diffraction maxima at 2.86 Å, approximately one third of the total amino acid residues as glycine, and a relatively high content of proline and hydroxyproline.

**Biosynthesis of Collagen.** One of the most important developments in the study of collagen biosynthesis was the discovery by Stetten\(^{20}\) that animals fed radioactively labeled free hydroxyproline did not incorporate it into collagen, although earlier reports had demonstrated that similarly labeled proline could act as a precursor to the hydroxyproline. This unusual finding indicated that the hydroxylation of proline was a key step in the biosynthesis of collagen. The parallel observation was also documented for hydroxylysine.\(^{21}\) Since then it has been found that the proline is incorporated into peptides before it is hydroxylated. Furthermore, it was demonstrated that the oxygen of the hydroxyproline hydroxyl was derived from *molecular oxygen* rather than from water\(^ {22, 23}\) and that this conversion is catalyzed by an oxygenase.\(^ {24}\) Still further observations indicated that in the absence of molecular oxygen a hydroxyproline-deficient protein was formed with a collagen-like characteristic—namely, it was degradable with the collagen-specific enzyme collagenase.\(^ {25}\) This material has been termed “protocollagen.”\(^ {26}\) Furthermore, the oxygenase (proline hydroxylase) has a specificity determined in part, by a peptide sequence. Udenfriend\(^ {27}\) has reviewed much of this work and presented a scheme for hydroxylation.

An interesting corollary is the mechanism acting in the biosynthesis of resilin, a rubberlike structural protein occurring in arthropods. Here the strength of this fibrous material is derived from cross links involving dityrosine and tri-tyrosine.\(^ {28}\) These amino acids appear to be formed through enzymic oxidation of tyrosine involving a peroxidase.\(^ {29}\) Furthermore the tyrosine which is first incorporated into polypeptide chains comes either directly from free tyrosine or from the hydroxylation of phenylalanine.\(^ {30}\) The latter requires an oxygenase, in this case phenylalanine hydroxylase. The important distinction from col-
lagent is that tyrosine can be incorporated by the organism directly, whereas hydroxyproline cannot.

An important question is whether or not a complete but unhydroxylated true collagen could be formed under certain circumstances and if so whether it could be aggregated into cross-linked macromolecular fibrils having desirable structural connective tissue properties. As far as the most reasonable structural models for collagen go there is no stereochemically significant reason why an unhydroxylated true collagen could not exist, but autoradiographic evidence has been presented to indicate that "protocollagen" accumulates only intracellularly and is then released for extracellular collagen synthesis.\textsuperscript{31} On the other hand, there are considerable data to indicate that the shrinkage and denaturation temperature (and hence stability) of various collagens decreases with a decrease in the total amino acid content\textsuperscript{28} which suggests that stabilization may be dependent on total pyrrolidine content. Considerations involving water content and hydrogen bonding\textsuperscript{23} between side chains of the triple helix further complicate the picture. Stability could also be provided through hydrogen bonding involving hydroxyproline\textsuperscript{44} and intramolecular cross links involving hydroxylysine.\textsuperscript{56} Regardless of the role of the imino acids in the stability of collagen or the factors involved in cross-linking, the significant point for the present is that synthesis of a complete, true collagen macromolecule can be specifically inhibited by the lack of molecular oxygen.

**Early Atmosphere and Collagen Formation.** Several studies, culminating in the most recent works of Cloud\textsuperscript{2} have indicated the importance of oxygen to the origin of the Metazoa. Most workers visualize development of photosynthetic organisms liberating free \( O_2 \) which in the earlier Precambrian was immediately demanded for inorganic reactions but which eventually reached a level "consistent with metazoan oxidative metabolism near the beginning of Paleozoic time." The critical concentration of oxygen is placed somewhere near 3 per cent of the present atmospheric level—less than 1 per cent oxygen. At this level the ozone absorption of lethal wavelength ultraviolet light becomes significant.\textsuperscript{4} This would have allowed a stepwise increase in phytoplankton in surface waters and therefore a concomitant stepwise increase in photosynthetic oxygen. For purposes of comparison with collagen requirements the apparent \( K_m \) (Michaelis constant) for \( O_2 \) in the proline hydroxylase system of the chick embryo is \( 3 \times 10^{-5} M \) or equivalent to 2.6 per cent oxygen.\textsuperscript{46}

The correlation between this scheme of atmospheric evolution and the biosynthesis of collagen is tempting. Inasmuch as the early metazoan stocks would be operating at minimal oxygen level, biochemical efficiency and economy would dictate physiological priorities. In referring to oxygenases in general, Hayashi\textsuperscript{27} has stated that they compete with the conventional electron pathways for oxygen and from a thermodynamic point of view "oxygenation reactions appear to be a waste of energy." Kaufman\textsuperscript{38} has similarly concluded that such aerobic hydroxylation reactions are "energetically expensive for the cell." I suggest, therefore, that in the early evolving organisms the much more efficient and physiologically necessary energy-generating reactions would have higher priority for the limited available molecular oxygen than would the formation of
dense fibrous collagen through the expensive enzymatic hydroxylation of proline and lysine. This would imply that primitive metazoans might have evolved with dense fibrous connective tissue restricted to high priority use. Such pre-conditioned and necessarily small organisms could then begin to synthesize the high strength structural collagen with its many adaptive advantages as soon as the oxygen level increased stepwise after the proliferation of uninhibited phytoplankton. Since collagen is evolutionarily primitive, occurring in every metazoan group, all then-existing metazoan stocks would be acted on and evolutionarily pressured by increased oxygen simultaneously and on a world-wide basis. This could result in the sudden appearance in the rock record of the Lower Cambrian of highly evolved and diversified organisms previously present in essentially unpreservable forms. Numerous polyphyletic origins in different groups would not be necessary.

Collagen Priority and the Fossil Record. When careful consideration is given to the early Cambrian faunas it is possible to conclude that functioning soft-bodied representatives of all groups are conceivable.1 2 39 The presence of a shell, cuticle, or carapace is not a mandatory prerequisite for the metazoan level of organization. In fact, it is a low priority physiological luxury, its functional significance notwithstanding.

The minimizing of connective tissue collagen as a major structural component would tend to inhibit development of all but the most incipient shell, cuticle, carapace, or the like. These protective devices are either made predominantly of collagen or are held together by collagen which acts as basement lamallae as well as serving to bind and connect muscle tissues. Furthermore, since normal muscle contraction requires oxygen to remove the products of anaerobic glycolysis (lactic acid accumulation), it would be uneconomical for an organism evolving under low oxygen levels to develop a significant musculature for metabolically expensive low priority physiological needs such as a shell.

Since a well-developed or enlarged musculature demands the fibrogenesis of collagen the early body wall muscles must have been weak and capable only of limited effects. This condition would effectively limit the organism's size, inhibit extensive locomotion, and would make burrowing impossible. This, in turn, would eliminate the major source of potential Pre cambrian trace fossils—tracks, trails, or burrows. Clark40 has discussed such locomotion and burrowing in terms of the necessity of development of an enlarged body-wall musculature in conjunction with a hydrostatic fluid skeleton. Both of these features are independent of a shell or carapace but both require significant formation of dense fibrous connective tissue.

The evolutionary utilization of collagen by primitive organisms in a low oxygen environment would be in accordance with their most immediate priorities. Very broadly, such needs would include the development of the coelom and enlarged musculature followed by segmentation. These requirements are necessary before the development of a shell or carapace could take place.40 It seems clear, by way of example, that the ancestral trilobites must have constructed a segmented coelom and musculature for a hydrostatic skeleton prior to the development of a rigid exoskeleton with its necessarily complex skeletal
molecules. While the latter has a very clear functional and adaptive significance it is a physiologically lower priority and energetically more expensive structure for an evolving organism faced with a limited oxygen budget. This is also true for thick cuticles and, above all, for shells. Since these external structures would reduce the available surface area for respiration they would be poor investments for primitive organisms lacking advanced circulatory-respiratory mechanisms or those living under near anaerobic conditions.

It is reasonable that primitive organisms in the Precambrian had evolved the preadaptive ability to secrete a necessarily thin shell or carapace, limited muscles, and the mechanism to synthesize the binding connective tissue. However, if the molecular oxygen necessary to catalyze this potentially eruptive adaptive break-through is competed for and channelled by physiological priority to more important uses, then the shell-carapace-muscle system would not be able to accumulate and develop to any but the most primitive degree for lack of the ingredients to hold it together, much less operate it. Nevertheless any development at all along these lines, however weak, would be of functional importance both for external protection and as a radiation shield. The sudden environmental change in atmospheric oxygen level would result in a dramatic shift from a clearly preadaptive condition, to a highly eruptive one. The idea of a preadaptive condition for collagen synthesis is reinforced by the observation that although specialized cells are usually associated with collagen formation there is increasing evidence that other cells can be stimulated to produce this protein. Further support can be derived from the efforts of Millard and Rudall. They were able to show that in earthworm cuticle the organization of collagen depends on vertical cell processes which may be related to "cilia" type structures. This, they interpret, may represent a primitive condition in the development of collagen as an organized extracellular structure. Rudall states: "If Metazoa derived from ciliated protozoan types these could have suffered a change to a collagen pellicle supported by cilia. It is not a great step to the modification of the cilia and their disappearance as motile organelles thus giving rise to separate cells linked together by a collagenous matrix, or to epithelia linked by a collagenous cuticle."

In this way the apparent contradictions presented by Cloud might be resolved. He could not visualize a trilobite and its complicated musculature without its associated carapace nor a brachiopod and its musculature without a shell. Since one is useless without the other, these organisms could have evolved with neither or with both in a much reduced state which was environmentally and physiologically regulated.

Among the diversified organisms those capable of first utilizing oxygen for extensive collagen fibrogenesis would likely have been among the most primitive. These would be those with the fewest organs, least demanding muscles or other energy requiring structures and more importantly, those lacking an advanced circulatory-respiratory system using instead oxygen exchanged through the epidermis by diffusion. Such oxygen might be more readily available for collagen synthesis than would be oxygen partitioned and competed for by a more advanced circulatory-respiratory system. This speculation is supported by the...
fact that the earliest known metazoan fossils (the Ediacaran fauna) are not those with “hard parts” but those with the hardest or most resistant “soft parts,” that is, those with major quantities of collagenous connective tissue. Included in this group are the coelenterates, the various types of worms, and the sponges.

In conclusion, I agree with those who believe that an increased atmospheric oxygen level “triggered” the proliferation of the Metazoa near the beginning of the Paleozoic but I disagree with the conclusion that there is no good reason why we have not found the records of Precambrian ancestors. If the records of Precambrian Metazoa (the stratigraphic arguments aside) are to be extended with finds similar to the famous Ediacaran assemblage the following words from Cloud\(^2\) are worth serious consideration by all paleontologists interested in this problem: “... so far as a substantial record of Precambrian life is concerned, we have mostly been looking for the wrong things, in the wrong rocks, with the wrong techniques...” It seems to me worth considering what various metazoan ancestors lacking protective coverings might have looked like, how large they might have been, and then, with an open mind and the appropriate tools, examining undoubted Late Precambrian sedimentary sequences for their remains.

The many preservational biases involved in this problem have been emphasized by Simpson.\(^1\) The statistics of preservation is a function of the organisms’ structure and composition and of the enclosing sediment type. Most importantly it is a function of the sedimentary processes involved. Catastrophic one-cycle, rapid burial in fine-grained sediments followed by isolation from further sedimentary disturbance for all subsequent time is mandatory for extensive assemblage preservation. At this point organism structure and composition become important. Nonarticulate and colonial sessile calcified organisms together with disarticulated hard parts of other organisms can survive more than one sedimentary cycle (pre- and postdepositional reworking). On the other hand, soft-bodied organisms and trace fossils cannot. The sedimentary bias is clear, as is the preservational hierarchy. A whole gastropod stands a better chance to become a fossil than a complete articulated crinoid, than a cuticled annelid, than a cellular, collagen-deficient animal of any type.

Note added in proof: Schopf and Barghoorn (J. Paleontology, 43, 111 (1969)) have argued that the Late Precambrian was more oxygenic than previously assumed (>1% present level). While, as they point out, this would place severe restrictions on the idea of the origin of the Metazoa being coincident with the beginning of the Paleozoic, it is still compatible with the developmental priorities expressed above.

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