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THE CROSS-β CONFIGURATION IN SUPERCONTRACTED PROTEINS

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SUMMARY

Models have been derived for the formation and lateral linkage of the transverse folds in the supercontracted state of the k-m-e-f group and other proteins. There are two solutions depending on whether the 180° bend is based on the so-called 2_7a fold or the 2_7b, and each requires certain sequences of amino-acid residues. Also discussed are the general features to be expected in X-ray diagrams, the elongation accompanying the transition from the cross-β to the parallel-β configuration, the "parallelism" or "anti-parallelism" of the aggregated chains, and the energetics of supercontraction.

INTRODUCTION

The property of supercontraction shown by the k-m-e-f group of fibrous proteins has long been thought of as a shortening of the α-configuration to some transversely-folded configuration[1], and this view eventually received its strongest support from the correlation of the phenomenon with the "cross-β" type of X-ray diffraction diagram[2], so called because the 4.65 Å reflection associated with the CO . . . HN linkage, which occurs on the equator in the "parallel-β" diagram produced by

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stretching keratin, myosin, etc., is found instead on the meridian. Supercontraction and the production of the cross-β diagram in keratin has now been a subject of investigation, by WOODS, WHEWELL, MERCER, SIKORSKI, PEACOCK[3] and others, on and off for many years, but over and above this it has attracted particular attention because of its apparent correspondence with muscle contraction[1]. As has often been explained, the idea is that muscle contraction is fundamentally only a special manifestation, in myosin, of the property of supercontraction common to all the members of the k-m-e-f group.

This interpretation by analogy became much more plausible and alive, so to speak, when a naturally-occurring example of the characteristic cross-β reflection was found in X-ray diagrams of bacterial flagella, where it is superposed on an α-pattern with macroperiod about 410 Å, like that of skeletal muscle, thus leading to the corollary that such flagella are a kind of monomolecular muscles[4,5]. And then a strikingly perfect example of a natural cross-β structure, which moreover readily undergoes an intramolecular transformation into the parallel-β configuration on stretching, was discovered in the egg-stalk of the lace-wing fly *Chrysopa*[6]; and more recently still has come the detection of cross-β effects in actomyosin by the action of adenosine triphosphate[7] and even of quite small changes in pH alone[8]. Altogether, supercontraction points quite clearly now to a new polypeptide configuration shorter than the α-configuration, and more than ever must it be reckoned with in trying to uncover the fundamental molecular event in muscle contraction. The question of the exact form of the supercontracted fold likewise becomes more pressing, the solution required being one which could apply to proteins in general—for example, stretched heat-denatured egg-white gave the prototype pattern[9]—but which achieves a special perfection in the structure of the egg-stalk silk of *Chrysopa*. With the help of appropriate models we have therefore taken up the question again in some detail, and the present communication sets out our findings for the general case.

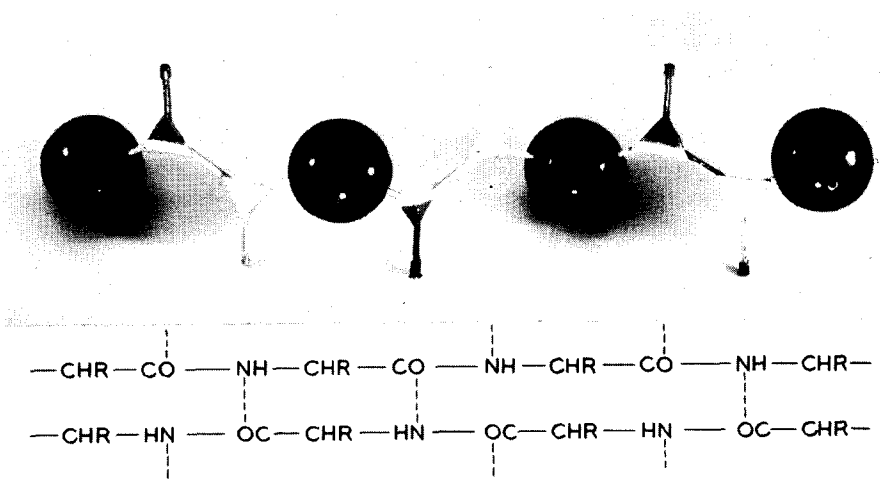

Fig. 1. Illustrating the rigid planar amide groups used in constructing the skeleton models. The short sequence shown forms part of the pleated-sheet β-configuration represented by the formula below.

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EXPERIMENTS AND RESULTS

For the final tests we have used the Courtaulds atomic models described by HARTLEY AND CONMAR ROBINSON[10] and applied to protein chains by CONMAR ROBINSON AND AMBROSE[11], but first we explored the possibilities with skeleton models constructed from rigid metal planar amide groups of the kind illustrated in Fig. 1. Their dimensions are those given by PAULING AND COREY[12], rotation is possible only around the bonds to the α-carbon atoms, and the hydrogen bonds between CO and NH groups are completed by means of short lengths of rubber pressure tubing as illustrated in Figs. 3 and 4.

Preliminary trials showed that when transversely-folded polypeptide chains lie side by side they can form excellent inter-chain (inter-bend) hydrogen bonds in the manner outlined in Fig. 2; in fact this appears to be the only satisfactory way in which regular lateral linkages can be formed. The hydrogen-bonded sheets thus generated can then be placed on top of one another to build up the third dimension by suitable side-chain linkages.

The transverse folds themselves and their internal hydrogen bonds have been taken to correspond to the anti-parallel pleated-sheet configuration, except of course at the 180° bends, which are in any case unobtrusive—they cannot bulge out like the heads of clubs, at least when linked up to form sheets as in Fig. 2—because they have little or no effect on the overall separation of the chains as measured by

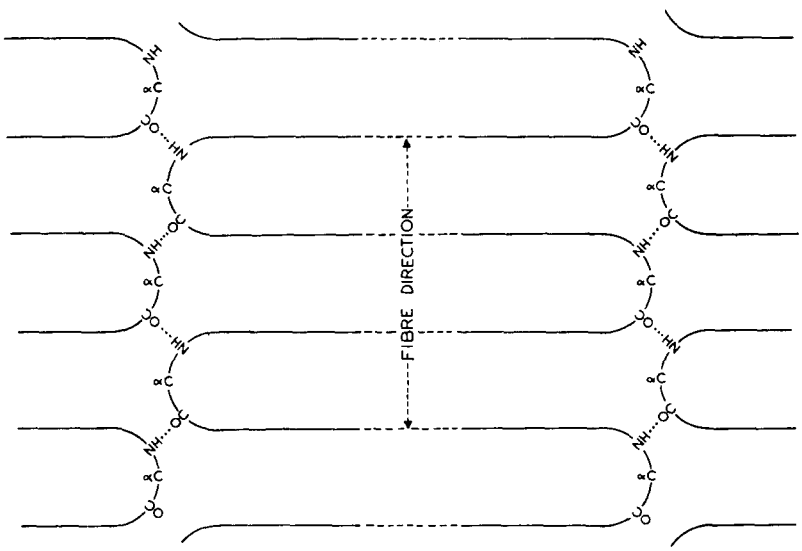

Fig. 2. Diagrammatic representation of the formation of hydrogen bonds between adjacent bends in transversely-folded polypeptide chains lying side by side.

the associated period along the fibre axis. This remains practically the same as the equatorial spacing of the parallel-β configuration, though PEACOCK[3] has reported detecting a small change with wool (from 4.65 Å on the equator of stretched wool to 4.71 Å on the meridian of supercontracted wool).

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The 180° bend must comprise a 7-membered ring of the type discussed by HUGGINS and others[13-21]. This 2_7 fold, as it was named by BRAGG, KENDREW AND PERUTZ[22], can be formed in two ways depending on the position taken up by the side-chain: in the original suggestion of HUGGINS, designated 2_7a, the side-chain stands out roughly normal to the plane of the ring, while in the alternative form, 2_7b, it lies roughly in the plane of the ring. The exact shape of the 2_7 fold is determined finally by the length and deviation from linearity of the hydrogen bond (for a full discussion see, for example, BAMFORD *et al.*[21]), and we have simply assumed about 2.8 Å for the N . . . O distance and a minimum angle (about 22°) between the NH bond and the N . . . O vector. A most important distinction between 2_7a and 2_7b, however, was pointed out by CONMAR ROBINSON AND AMBROSE[11]: it is at once clear from a Courtaulds model that a polypeptide chain can easily be folded back on itself provided the fold formed at the bend is 2_7b, but the operation appears to be extremely difficult if not impossible when one tries to form 2_7a in such a way, because of steric hindrance from the side-chain at the apex of the bend. The obstruction is removed when the apical side-chain is only the hydrogen atom of a glycine residue, in which case 2_7a can be formed just as readily as 2_7b.

It follows, therefore, that for regular transverse folds in general the 180° bends to be expected will be of the type 2_7b, but if the apical residue is glycine, then 2_7a also is permissible.

Continuing now away from the bend along the body of the transverse fold, we have found as many as sixteen conceivable variations in the sequence, but all except one for 2_7a and another for 2_7b are eliminated by steric hindrances of various kinds, either within the transverse folds themselves or when the inter-bend linkages indicated in Fig. 2 are attempted. A skeleton model of the remaining terminal configuration in the general case based on 2_7b and L-residues is illustrated in Fig. 3(b), while the counterpart configuration based on 2_7a is illustrated in Fig. 3(a). In these models the bonds to the β-carbons of the side-chains are represented by short pins, and they are shown projecting from the apical α-carbons in both pictures; but this is merely to bring out the difference between 2_7a and 2_7b, no actual β-carbon, but only a hydrogen, being likely in the side-chain direction at the apex of 2_7a.

Comparison between the skeleton models constructed as in Fig. 3 and corresponding Courtaulds models revealed an interesting point with regard to flexibility and the ease with which transverse folds can be formed, in that when the skeleton chain is folded back on itself the second intra-fold hydrogen bond (the next after the 7-membered ring at the bend) does not form automatically in either case at a bond-length of about 2.8 Å. With the metal amide units of the dimensions specified[12] and linked simply by insertion into appropriate holes drilled in wooden α-carbon atoms, the minimum "free", unstrained N . . . O distance appears to be perhaps as much as 3.9 Å, and it is necessary to press the arms of the fold together in order that the piece of pressure tubing may be fitted to hold the bond at 2.8 Å. No such "trouble" is experienced, or at least is scarcely noticeable, with the Courtaulds polypeptide chain, because the rubber washers incorporated in the covalent bonds (except in the fixed flat amide groups) permit variations in the bond angles of up to 6°, with the result that there is a ready distribution of strains which leaves the impression that their overall significance is unimportant.

Continued repetition of the transverse folds leads to long ribbons in what it is

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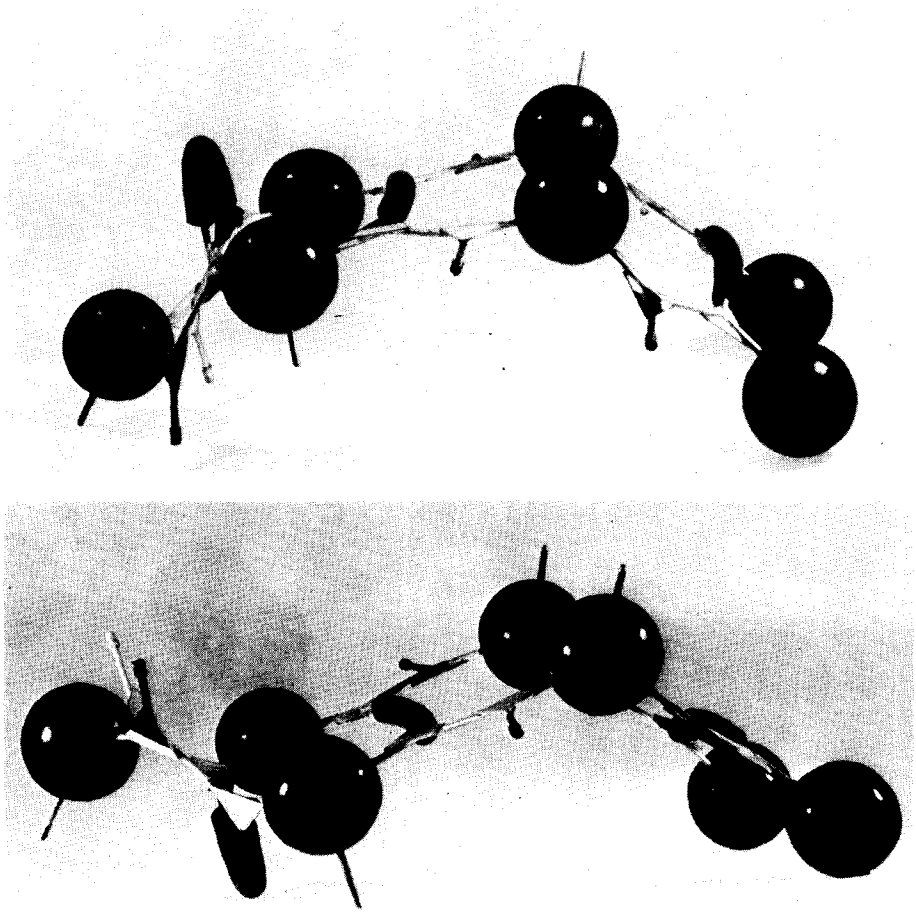
useful to call the "jumping-cracker" (or "Chinese-cracker") configuration running, when oriented by stretching, in the direction of the fibre axis, with a minimum fibre period of twice the hydrogen-bonded separation of the chains (that is, twice the "backbone spacing" in the original k-m-e-f phraseology). For this to come about,



(a)

(b)

Fig. 3. Skeleton models of the terminal configurations of transverse folds based on (a) 2_7a, and (b) 2_7b. The amino-acid residues are in the absolute L-configuration, and the short pins represent the directions of the bonds to the β-carbons of the side-chains. Note, however, that most probably the "side-chain" at the apex of 2_7a can only be a hydrogen.

the number of residues (n) lying between one bend say on the left and the next on the right must be *odd*, and the contour of the transverse folds as viewed along the length of the "jumping cracker" is of the "chair" form. If like bends at opposite ends of a fold are made after an even number of residues, the result is the "boat" form and the chain turns back on itself in the same rotatory sense, leading to the possibility of a cyclic peptide, or just conceivably, with certain distortions, to a laterally-flattened spiral with the side-chains pointing along the fibre axis.

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When the "jumping crackers" lie side by side and are hydrogen-bonded as illustrated for 2_7a in Fig. 4 (in which the inter-bend hydrogen bonds are marked B), they give rise to "stepped" sheets in which the steps run lengthways. The steps are slight in the case of 2_7a, being hardly more than 1 Å high, but they are as much as about 8 Å high in sheets built from 2_7b. On looking along the "jumping crackers" in sheets built from 2_7a, it is seen that the apical α-carbons are practically in register (that is, lie in planes standing parallel to the steps and perpendicular to the sheet); while in sheets built from 2_7b the step is S-shaped and the α-carbons flanking the apical α-carbon in one bend are almost exactly in register with the flanking α-carbons in the two bends to which it is hydrogen-bonded. This peculiarity of the 2_7b sheets means that, dimensionally, there is a pseudo-continuity in the polypeptide chains on passing from one "jumping cracker" to the next, which could make it appear, in certain aspects of X-ray diagrams, that the amino-acid sequence in the transverse direction is uninterrupted; in other words, that there are *continuous* long chains lying perpendicular to the fibre axis. In any event, the overlap means also that the effective structural length of the transverse fold as inferred from sufficiently perfect cross-β diagrams will correspond in this particular case not to the full n residues between successive bends but to $(n-2)$. On the other hand, the effective structural length of the transverse fold in 2_7a sheets will correspond closely to the distance between apical α-carbon atoms. Both these coincidences, however, hold only for models constructed as in Fig. 4, in which the inter-bend hydrogen bonds are linear and of length about 2.8 Å. If, for example by reason of steric requirements associated with the side-chain linkages, these conditions are departed from, variations in the lateral separation may be expected.

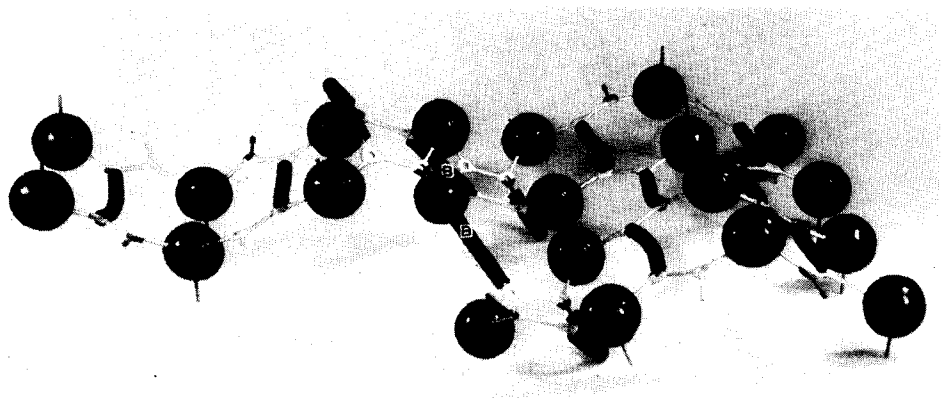

Fig. 4. Skeleton model of the hydrogen-bonded stepwise union between the bends in one upper and two lower transverse folds in the 2_7a configuration. The inter-bend hydrogen bonds are marked B.

The theoretical elongation accompanying the complete intramolecular transformation from the cross-β to the parallel-β configuration should be about $(n \times 3.5)/4.7$. For $n = 9$, for example, this comes to about 6½, which is roughly what is observed

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with the *Chrysopa* egg-stalk. With keratin and myosin the observed elongation from fully supercontracted to fully extended falls also within the same kind of range —say from four to five times the supercontracted length—suggesting five or seven residues from one bend to the next.

Considering next the question of how transverse-fold formation and linkage may depend upon amino-acid sequence, the models show further, especially the Courtaulds models, that there are indeed other limitations besides the one already mentioned that the apical residue for 2_7a can most probably only be a glycine. One of the two residues flanking the apex of the 2_7a bend must also be a glycine, though the other may have any side-chain; and this holds whether or not the 2_7a "jumping cracker" is hydrogen-bonded to neighbours as part of a sheet. (It seems pertinent to include, too, the case of "free" chains, which have satisfied their spare hydrogen bonds at the bends by combination with smaller accessory molecules, and which in themselves could very well be of sufficient size and regularity to give rise to recognisable cross-β diffraction effects.) Sheets built from 2_7b can accommodate residues other than glycine at the apex of the bend, but then the two flanking residues must *both* be glycines. (In the special case where the apical residue happens to be a glycine, one of the two flanking residues must still be a glycine but the other can be an alanine.) The permissible residues at the apex, according to our tests, are: glycine, alanine, serine, cysteine, valine, threonine, leucine, isoleucine, and aspartic acid; and we also came to the conclusion that it was just possible, with a little strain, to incorporate a half-cystine residue and link it up with the other half in one of the transverse folds in the adjacent "jumping cracker"—a particularly interesting finding which suggests that the supercontracted state may be reached without necessarily breaking all the disulphide bridges between chains cross-linked by cystine. When 2_7b "jumping crackers" are not bonded into sheets, they can have any residue both at the apex and at one of the flanking positions, but the other flanking residue must still be a glycine.

These results for the sterically permissible sequences at the bends may be summarised as follows, in which the apical residue is enclosed within brackets, R stands for any kind of amino-acid residue, and X stands for one of the residues of glycine, alanine, serine, cysteine, valine, threonine, leucine, isoleucine, aspartic acid, and ½-cystine:

2_7a (hydrogen-bonded)	–R–(gly)–gly–
2_7a (free)	–R–(gly)–gly–
2_7b (hydrogen-bonded)	–gly–(X)–gly–
	–gly–(gly)–ala–
2_7b (free)	–R–(R)–gly–

Finally, it is important to give some discussion to the rather curious problem, as it turns out, of the so-called "parallelism" or "anti-parallelism" of the aggregated polypeptide chains in the cross-β and parallel-β states; for it will be seen from Fig. 2 that side-to-side hydrogen-bonding in the cross-β configuration proposed here—and we have found no convincing alternative in our studies of the models, at least if it is desired to build up combinations with any crystallographic regularity—requires chains running in the direction of the fibre axis all in the same sense, that is in *parallel* array. Yet it has long been known[23], ever since the discovery of the α–β transformation, that in the parallel-β diagram given by stretched keratin (and by other members of the k-m-e-f group, and by heat-denatured egg-albumin stretched in

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steam) there is a clear reflection on the first layer-line to which we assigned the indices (111) and from which we inferred that the full "backbone spacing" is not 4.65 Å but 2×4.65 Å; in other words, that there is an alternation of chains which are crystallographically dissimilar and therefore most probably in *anti-parallel* array[24,25]. PAULING AND COREY, however, in their latest study of possible "pleated sheets" for β-structures[26], have shown that both a parallel and an anti-parallel form can be constructed with practically linear hydrogen bonds, the former leading to an estimated axial repeat of 6.5 Å and the latter to 7.0 Å (with $a = 4.85$ Å and 9.5 Å, respectively); and on this basis they have proposed the parallel-chain form for the β-keratin type of structure and the antiparallel-chain form for silk fibroin. While this choice is consistent enough with the observed axial repeats (about $3^1/_3$ Å for β-keratin and $3\frac{1}{2}$ Å for silk fibroin), it would conflict as it stands with the (111) interpretation of the spot on the first layer-line of the parallel-β keratin diagram, and we have accordingly examined the PAULING-COREY models again with a view to resolving the paradox. The solution we suggest is this: that while the main-chains considered alone are in parallel array, *the side-chains attached to them project in an anti-parallel arrangement.* On alternate main-chains they are deflected in contrary directions, lying forwards along one chain and backwards along the next. This would account very simply and reasonably for the fact that the β-keratin structure is adopted by fibrous proteins (excluding the collagen group) with many bulky side-chains, as opposed to fibroin, in which the biggest side-chains are mostly those of alanine and serine. They can find sufficient room by pointing away from one another, and there is in addition a slightly increased *a*-dimension.

A fuller discussion of this re-assessment of the β-keratin diagram, and of a re-examination of the transition between the cross-β and parallel-β states in heat-denatured egg-albumin[27], will be published later. For the present we wish to make the point that, with the k-m-e-f group and heat-denatured egg-albumin, the parallel-β as well as the cross-β state appears now to be based on at least main-chains in parallel array, and therefore a change-over from one to the other can take place by means of a direct intramolecular transformation. It is unnecessary though, and would be unjustified, to expect this direct correspondence always, since the argument applies only to individual sheets. The chains in alternate sheets, as they are piled on top of one another and linked by their side-chains, can run in opposite directions, or there may be a random distribution of sheet-directions or of crystallite-directions, or both; and a change-over from parallel to anti-parallel array could thus take place by means of a re-pairing of chains.

The energetics of the interchange between α and supercontracted also raises questions that call for analysis, but the data are insufficient yet for anything beyond drawing attention to: (1) DONOHUE's estimate[28] that there is an instability of the order of 0.5 kcal. associated with the formation of the 2_7 fold, which, however, would be negligible when related to a transverse fold as a whole, and could be of less significance still against the background of unknown side-chain interactions; and (2) the analogy between the cross-β configuration in proteins and the regularly-folded configuration discovered by KELLER in crystallites of polyethylene and other synthetic chain-molecules[29]. The driving force for such a preference in these simplest-possible, chemically inactive chains of polyethylene must be purely entropic, and a similar tendency is to be looked for in polypeptides too, in addition to internal energy effects

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and the likelihood of special fold formation by virtue of many active side-chains. In general, longer and longer *straight* chains will become increasingly less probable, and shorter straight sections may be expected to be incorporated in transverse folds whose optimal length may finally be decided by more specific chemical considerations. However that may be, it is an experimental fact that muscle and myosin, for example, prefer to shorten from the α-helical configuration to the transversely-folded cross-β configuration just as soon as the temperature of the water in which they are immersed is raised above only about 40°.

Further investigations into the structure of the *Chrysopa* egg-stalk are continuing and will be the subject of a separate communication[30].

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