Comparative Sequence of Myosin Light Chains from Normal and Hypertrophied Human Hearts

CATHERINE KLOTZ, JEAN J. LÉGER, AND MARSHALL ELZINGA

SUMMARY Myosin light chains from normal and hypertrophied human hearts were partially sequenced in order to see whether structural modifications of these subunits could provide a molecular basis for the changes observed in heart properties and in myosin enzymatic activity. Normal light chains were prepared from hearts taken at autopsy, weighing 350 g or less and apparently devoid of myocardial disease. "Hypertrophied cardiac myosin light chains" were prepared from two greatly hypertrophied hearts, weighing 600 and 750 g. No amino acid substitutions, deletions, or additions were observed in the light chains from hypertrophied hearts. The third light chain previously reported in human cardiac myosin and related to hypertrophy was found to be a proteolytic product of LC2. The comparison between human and beef cardiac myosin light chains indicates that the sequences of these subunits of the myosin molecule are highly conserved. Circ Res 50: 201-209, 1982

CARDIAC hypertrophy induced by chronic pressure overload leads to an impairment of mechanical performance of the heart (Hamrell and Alpert, 1977) with a simultaneous decrease of the specific activity of myosin (Swynghedauw et al., 1973; Raskowski et al., 1977; Carey et al., 1979). In contrast, increased contractility and increased myosin ATPase activity are observed in cardiac hypertrophy induced by thyrotoxicosis (Yazaki and Raben, 1975; Banerjee et al., 1976). It seems likely that the observed changes in myosin's enzymatic activity, and the changes in the properties of the heart muscle, result from changes in the constituent myosin, which could in principle involve either alteration of the normal myosin (e.g., methylation, phosphorylation, or other post-translation change), or the synthesis of a different "isozyme" of myosin. Synthesis of a new form of any one of the three types of chains present in the myosin could cause an alteration of the enzymic properties of the myosin. Various investigators have examined the myosin of hypertrophied hearts, and it is now clear that the number and size of the subunits do not change (Swynghedauw et al., 1973; Katagiri and Morkin, 1974; Klotz et al., 1975; Malhotra et al., 1977). Chemical analyses have given no indication of post-translational modifications, but they are not ruled out by the evidence at hand. Flink and Morkin (1977) and Flink et al. (1979) have presented CNBr peptide maps which show apparent differences in pattern between the heavy chains of normal cardiac myosin and myosin from thyrotoxic hearts, while Schwartz et al. (1978) have shown that mechanical overload leads to synthesis of an immunologically distinct myosin. Recent studies by Hoh and Egerton (1979) and Lompré et al. (1979) involving electrophoresis of myosin under non-denaturing conditions suggest that the relative proportions of myosin isozymes can change under stress conditions, but these studies cannot distinguish whether the molecular basis for variation in electrophoretic properties involves new heavy chains, new light chains, or post-translational modifications.

The work reported in this paper is focused upon the light chains of cardiac myosin, and specifically we address the question of whether the light chains of myosin from human hypertrophied hearts differ in amino acid sequence from those of normal hearts. The rationale is that any difference, even if functionally insignificant, would prove that the heart can synthetize different forms of myosin under stress; on the other hand, no differences between the light chains would indicate that the same light chain genes are being expressed. Since in other muscles the light and heavy chains always appear to be matched, synthesis of the same light chains to associate with a new type of heavy chain would represent an arrangement not previously observed, except perhaps in the thyroxic heart (Flink and Morkin, 1979).

Since analytical techniques, such as SDS gel electrophoresis and total amino acid composition, do not show differences between light chains of myosin from normal and hypertrophied hearts, the only approach that proves unequivocally whether...
or not new chains are being synthesized is amino acid sequencing. The study reported here on myosin light chains isolated from normal and chronically overloaded human hearts uses this technique.

**Methods**

**Materials**

Human hearts were obtained from the anatomy pathology department of several Parisian hospitals, where the cadavers were put in the cold room within 1–2 hours after death. In the present work, all the autopsy material was obtained within 8–12 hours after death; the structural integrity of the molecules in these autopsy tissues was confirmed by electrophoresis on SDS gels. As noted in a previous study (Klotz et al., 1975), no effect on the overall structure or on the activity of the myocardial myosin is apparent up to 2 days after death.

We have designated as “normal” those hearts weighing 350 g or less and coming from patients with no known cardiac disease. In addition, each heart was autopsied and removed from this group if any myocardial injury was detected. We designated as “hypertrophied” those hearts weighting more than 550 g. In the present work, two hypertrophied hearts were used. The first patient (heart weight, 600 g; degree of hypertrophy, 170%) had hypertension and the autopsy showed a lateral myocardial infarction. The second patient (heart weight, 750 g; degree of hypertrophy, 220%) had mitral insufficiency. The autopsy showed a posterior myocardial infarction. All hearts used came from adults.

Beef hearts were obtained from a slaughterhouse 6–8 hours after death.

Beef hearts and human myocardia were sometimes frozen in liquid nitrogen and kept at −20°C until used.

**Preparation of Proteins**

Myosin was prepared from left ventricles according to the method of Swynghedauw et al. (1973). The light chains (LC) were dissociated from myosin by treatment with 6 M guanidine hydrochloride and alkylated by iodoacetic acid at room temperature (Elzinga, 1970). They were separated from heavy components by ethanol precipitation (Perrie and Perry, 1970) and concentrated by rotary evaporation.

The light chains isolated from human cardiac myosin were separated according to their molecular weights in 6 M guanidine hydrochloride by filtration on agarose beads (Biogel A 1.5 m, 100–200 mesh, 2 × 720 cm) (Klotz et al., 1978). The beef cardiac light chains were separated according to charge by chromatography on DEAE-Sephadex A-25 (Wikman-Coffelt et al., 1973).

**Gel Electrophoresis**

Isoelectrofocusing (IEF) was performed according to the method of O’Farrell (1975). The ampholyte (LKB) of pH range 3.5–10, 3–5, and 5–7 were mixed in the ratio 1:2:2 in order to have a linear pH curve from 4.5 to 7.0. Staining was performed according to the method of Weber and Osborn (1969) after gel fixation (Vesterberg, 1977).

Sodium dodecyl sulfate (SDS) polyacrylamide slab gel electrophoresis was performed according to the procedure of Porzio and Pearson (1977). Two-dimensional gel electrophoresis was, as usual, a combination of both techniques mentioned above but the isoelectrofocusing gels were systematically stained. If used for a second electrophoresis, the whole stained gel or part of it was put in a buffer containing SDS (buffer “0” from O’Farrell (1975)) for 30 minutes. The gel pieces were then applied on the top of the SDS slab gel and electrophoresis was performed as usual. Under these conditions, no protein remained in the IEF gel after the second electrophoresis and no difference was observed in the electrophoretic pattern of LC whether or not the IEF gel was previously stained.

**Methods of Cleavage and Fractionation of Peptides**

Each pure light chain was cleaved at methionine residues by cyanogen bromide (1000-fold molar excess) treatment in 70% formic acid (room temperature, 16 hour).

The peptides were applied to a Sephadex G50 (superfine) column (2 × 400 cm) in 25% acetic acid. This filtration permitted the isolation of some pure peptides which were sequenced (see Results).

**Amino Acid Analyses**

Amino acid analyses were carried out on samples hydrolyzed for 22 hours in 6 N HCl in evacuated sealed tubes. Cysteine was determined as carboxymethylcysteine and methionine as homoserine. Relative recoveries of methionine and homoserine gave an indication of the cleavage efficiency.

**Sequence of Peptides**

Amino acid sequences were determined using a Beckman 890C automatic sequencer; in general, the 0.1 M Quadrol program was employed. Identification of the PTH derivatives was based upon thin layer chromatography, amino acid analysis following hydrolysis of the PTH amino acid (6 N HCl, SnCl₂, 150°C, 4 hours) or high pressure liquid chromatography.

Carboxylic acid-terminal sequence analyses of the intact proteins and peptides were done by digestion with carboxypeptidase A or B (Worthington). The amino acids removed were identified and quantified by automatic amino acid analyses.

**Results**

**Electrophoretic Analysis of the Light Chains**

Myosin from normal and hypertrophied human hearts, prepared under our experimental conditions,
contained two light chains of molecular mass 25,000 daltons (LC1) and 19,000 daltons (LC2) (Fig. 1a). One additional 15,000 dalton polypeptide chain copurified with the myosin and was present in the same proportion in all preparations of human heart myosin (Fig. 1a). Because its behavior was related to that of the light chains LC1 and LC2, it has been named LC3 (Klotz et al., 1975). This chain was isolated and further sequenced along with the other light chains. Myosin from bovine hearts contained two light chains of molecular mass 26,000 (LC1) and 19,000 (LC2) daltons (Fig. 1d).

On two-dimensional gel electrophoresis, LC1 isolated from normal or hypertrophied human hearts or from bovine hearts gave one spot whereas LC2 gave two spots (Fig. 1, b and e). These two spots of the same molecular weight corresponded to a charge heterogeneity which could be attributed either to the coexistence of phosphorylated and unphosphorylated forms of this LC2 (Perrie, 1973) or possibly to partial deamidation of Asn or Gln residues. The additional human LC3 gave one spot suggesting the homogeneity of this protein in respect to its molecular mass and charge. Experiments in which myosin from normal and hypertrophied human hearts were mixed and run together confirmed their identical behavior. Results concerning the comparative stoichiometry of these light chains were previously published (Klotz, 1975) and did not indicate any difference.

Isolation of the Light Chains

Two factors limited the amount of light chains recoverable from human hearts. First, the available human myocardia were never complete because of autopsy sampling. Second, the quantity of purified myosin prepared from human hearts was lower than that available from other animal hearts. This low yield (2-3 mg myosin/g human myocardium) was probably due to the amount of fat in the human myocardium. In the case of normal human hearts, several autopsy pieces were pooled; about 50 mg of light chains were prepared and separated by gel filtration on Biogel A 1.5 m. One micromole of each pure light chain from normal cardiac myosin was used for sequencing.

With the hypertrophied hearts, no pools were made. In both cases studied, about 200 g of hypertrophied ventricles gave 15 mg of the mixed light chains. This allowed us to obtain about 200 nmol of each light chain for sequencing.

From 250 g of bovine left ventricle, one μmol of each pure light chain was prepared.

The purity of each isolated light chain was tested by electrophoresis (Fig. 1, c and f). Amino acid compositions of the three myosin light chains from normal human hearts were previously published (Klotz, 1975). Analysis of the amino acid compositions of the three myosin light chains from both hypertrophied human hearts revealed no differences from those of normal hearts.
Isolation of Peptides

Because of the low quantity of pure light chains available from one hypertrophied human heart, we attempted to use only gel filtration for isolation of the cyanogen bromide peptides. This method gives nearly quantitative recoveries, while the other methods available, such as ion exchange chromatography, result in significant losses. Therefore the peptide purification was limited to one filtration on a Sephadex G50 column.

Each of the three light chains contained seven methionines; the cyanogen bromide cleavage led therefore to the production of eight peptides from each light chain.

Human Cardiac Myosin Light Chain LC1

The elution pattern of the cyanogen bromide peptides from LC1 isolated from normal heart myosin showed eight well-defined peaks, each of which contained primarily one peptide. The first peak contained the N terminal peptide of LC1 and was eluted immediately after the void volume (600 ml); the last peak containing a dipeptide was eluted in the total volume of the column (1200 ml). The yields were essentially quantitative (Fig. 2, upper part).

The elution pattern of the cyanogen bromide peptides from LC1 isolated from both hypertrophied hearts also showed eight well-defined peaks...
eluted at the same volume as those from normal heart myosin LC1. The same pools were made.

**Human Cardiac Myosin Light Chain LC2**

The elution pattern of the cyanogen bromide peptides from LC2 isolated from normal heart myosin showed six peaks. The first one, eluted near the void volume (650 ml), contained a mixture of peptides. The following three peaks (690, 740, and 800 ml) were well defined, and each of them contained a pure peptide. Finally, a double peak emerged from the column between 870 and 940 ml, the ascending and descending part of which contained mixtures of peptides (Fig. 2, lower part).

The elution pattern of the cyanogen bromide peptides from LC2 isolated from both hypertrophied human heart also showed six peaks eluted at approximately the same volumes as those from normal heart LC2.

**Human Cardiac Myosin Light Chain LC3**

The elution pattern of the cyanogen bromide peptides from LC3 isolated from normal human hearts showed six peaks, which had the same elution volume and shape as those from LC2: the first one, eluted at 650 ml, contained a mixture of peptides, whereas the following three peaks (690, 740, and 800 ml) were well separated and contained pure peptides which were sequenced. As with those from LC2, the yields were very high because of the good separations and ranged usually between 50 and 70%. Finally, a double peak emerged from the column between 870 and 940 ml, the ascending and descending part of which contained mixtures of peptides.

The elution pattern of the cyanogen bromide peptides from LC3 isolated from hypertrophied human hearts showed six peaks eluted in the same volume as those from normal heart LC3.

**Beef light chain peptides were also isolated by**

### Table 1 Partial Amino Acid Sequence of Human Cardiac Myosin Light Chain LC1 Isolated from Normal Human Hearts (capital letters) and from Both the Hypertrophied Human Hearts (underlined residues)

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Comparison is made with bovine cardiac myosine LC1 (below the capitals) and with rabbit skeletal muscle myosin light chain Al (italic letters). The 50 first amino acids corresponding to the N terminal proline-rich region of skeletal LC Al was not drawn. Dotted lines underline the bovine residues corresponding to the part of the sequence previously published (Weeds, 1975) and modified during this study. Boxed residues mark amino acid replacements between human and bovine light chains. ?’s designate positions that were not identified.
gel filtration as previously described (Leger and Elzinga, 1977).

Detailed elution patterns and amino acid compositions of the peptides will be published elsewhere.

Sequence

Each human cardiac peptide that was sequenced was compared with the known sequences of the skeletal muscle myosin light chains and could, by homology, be unequivocally situated along the chain. Because of the extensive similarities, peptides of human LC were ordered and numbered by comparison with the sequences of the skeletal muscle myosin light chains (Frank and Weeds 1974; Collins 1976; Matsuda et al., 1977) (Tables 1 and 2).

Human Cardiac Myosin Light Chain LCl

From the eight peptides isolated from LCI, seven peptides with free-NH₂ terminals were sequenced. Their sequences, which corresponded to about 75% of the whole molecule, were determined using peptides from normal myocardia, and, independently from both preparations from hypertrophied myocardia. No amino acid substitutions, deletions or additions were observed between the LCI's from the normal and hypertrophied hearts. The peptide which was not sequenced had a blocked N-terminus and was further digested. The sub-peptides have not yet been completely sequenced. However, their amino acid compositions and partial sequences did not reveal any differences between the normal and hypertrophied heart preparations. Table 1 presents the sequences of the seven peptides from normal and hypertrophied human cardiac LCI.

A comparison of the sequences of human cardiac LCI peptides with the sequence of the light chain A1 from rabbit skeletal muscle myosin was made. The seven human peptides could be aligned by analogy with the skeletal light chain sequence and spanned the region 56 to 190 (Table 1). Twenty-five amino acid substitutions, distributed along the polypeptide chain, were found between the two types of light chains. One of these substitutions could be of interest because it involves the change of the hydrophobic residue Val 71 to Cys. Two additions

Table 2 Partial Amino Acid Sequence of Human Cardiac Light Chain LC2, Isolated from Normal Hearts (capital letters) and from Both the Hypertrophied Hearts (underlined residues)

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Comparison is made with rabbit skeletal muscle light chain LC2 (= DTNB-LC) and with partial sequences of beef cardiac light chain LC2 (below the capitals).

Residues 1 to 10 and 56 to 84 were not sequenced. '?'s designate positions that were not identified.
were observed: a dipeptide Pro-Lys was inserted between residues 61 and 62 and one Lys was added after residue 143. The human peptide with its NH₂ terminus blocked (the N terminal peptide of LC1) corresponded to the region 1 to 56 but, as already noted, alignment of partially sequenced peptides from the heart has been difficult. It may be that this peptide is highly specific for a particular muscle type.

The comparison of the sequences of the human and bovine cardiac (Weeds, 1975) LC1 peptides revealed some discrepancies and required the sequencing of three peptides from bovine LC1. This led to a rearrangement of regions: 44-65 [regions A1-S1 of Weeds (1975)], 96-102 (S2), and 160-165 (A4). We found a cyanogen bromide peptide beginning at residue 65; therefore residue 64 must be Met instead of Ser and the peptide previously placed at positions 59 to 65 was fitted at positions 96 to 102. Met 96 then was placed at position 104. The thiol peptide ordered at positions 44 to 52 overlapped the peptide 65 to 104 and therefore was placed in positions 59 to 65. Finally, the peptide 144 to 159 was found to be longer, spanning 144 to 167 since residue 160 was Thr instead of Met (Table 1). After these modifications, the comparison between human and bovine cardiac LC1 (positions 56 to 190) showed only four substitutions. This underlined the fact that species variations in equivalent muscle proteins from a given tissue are generally very limited.

**Human Cardiac Myosin Light Chain LC2**

The three pure CNBr peptides from human cardiac LC2, isolated by Sephadex G50 filtration, have free NH₂ termini and were sequenced without further treatment. Their sequences, which correspond to about 70% of the molecule, were established using peptides from normal as well as hypertrophied myocardia. Two other mixed peptides were analyzed on the sequencer, allowing us to establish their partial sequences. These last analyses were made only on peptides from normal hearts. The intact LC2 light chain was refractory to Edman degradation, presumably because of a blocked NH₂ terminus.

From the sequenced peptides and from the amino acid compositions of those which were not sequenced, no substitutions or deletions were seen between the LC2 light chain from normal or hypertrophied hearts. The alignments of all completely or partially sequenced peptides from human cardiac LC2 were made easily by reference to the sequence of the skeletal muscle light chain LC2 or DTNB-LC (Collins, 1976) and Table 2 presents the partial sequence of the myosin LC2 light chain from normal as well as hypertrophied human hearts. The completely sequenced peptides of human LC2 spanned regions 36 to 55, 85 to 120, and 139 to 165.

Microheterogeneities were found in positions 96 and 99 of the peptide spanning the region 86 to 123 of the sequence and in position 156 of the peptide scanning the region 140 to 168. These three heterogeneous positions were also found in the equivalent peptides from both hypertrophied hearts. In addition the ratios of these heterogeneous amino-acids were always about to 1:1.5. Table 3 summarizes these results.

Comparison between the skeletal and the human cardiac protein showed 26 amino acid substitutions distributed along the polypeptide chain. The presence of deletions in position 70 (Met) and 92 (Lys) should be noted. The essential amino acids of the putative calcium binding site in LC2 (Collins, 1976) are all conserved.

The comparison between human and bovine cardiac LC2 was limited because, so far, few data have been published on beef LC2 (Leger and Elzinga, 1977). Comparison with the already sequenced peptides (85 to 95, 123 to 154), which represent about 25% of the molecule, showed one change in position 95 in the two species: Asn (bovine) → Asp (human). No positions corresponding to the human LC2 microheterogeneities have been sequenced in bovine light chain.

**Human Cardiac Myosin Light Chain LC3**

The three pure peptides, isolated by gel filtration of the CNBr cleaved human cardiac LC3, from normal and hypertrophied hearts had amino acid compositions and sequences that were identical to the three peptides isolated from human cardiac LC2. Even the same microheterogeneous positions with the same ratios were found as in the corresponding peptides from human cardiac LC2. Edman degradation applied to the whole LC3 indicated that it had a free NH₂ terminal amino acid, in

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<td>Asx (40%)</td>
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<td>Thr (60%)</td>
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<td>Leu (40%)</td>
<td>Leu-His</td>
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<tr>
<td>165</td>
<td>Lys-Asx</td>
<td>Val (60%)</td>
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contrast to all other myosin chains. Sequence analyses of the entire chain gave, for each step, a mixture of two amino acids in a ratio 1:3, indicating that the chain was cleaved at two points: between Ala 9 and Ala 10, and between Ser 14 and Ser 15. It was thus assumed that LC3 is generated by the proteolysis of the LC2 light chain. The same conclusions were drawn from the sequence studies made on the LC3 isolated from both hypertrophied hearts.

**Discussion**

The work reported here represents the first direct comparison of the amino acid sequences of myosin light chains from normal heart with those from hearts that may be considered to be abnormal. The designation is based upon the fact that the hearts are abnormally large (about twice normal), and clinical observations. One case is an example of pressure hypertrophy (arterial hypertension) and the other represents a volume hypertrophy (mitral insufficiency), both being associated with a coronary disease.

In experimental pathology, it has been demonstrated that such heart hypertrophy lead to changes in the myosin molecule. After chronic mechanical overloading due to pressure and/or volume increase, the heart enlargement is accompanied by a decrease of the Ca-ATPase activity of myosin (Swynghedauw et al., 1973; Scheuer and Bhan, 1979), a change in the isozymic pattern of myosin (Lompre et al., 1979), and a modification of the heavy chain structure (Flink and Morkin, 1977; Siemankowski and Dreizen, 1978). The absence of complete sequences leaves open the possibility that amino acid substitutions between the light chains from normal and hypertrophied hearts may have been missed in the portions that we have not sequenced. However, we think that the similarities of the amino acid compositions and partial sequences of the portions remaining to be sequenced suggest that they are also identical with the normal light chain. We suggest therefore that conclusions drawn from the completed sequence are likely to be valid for the entire chains, viz., that the same light chains genes are being expressed in both normal and hypertrophied cardiac tissue.

The sequence studies on the human extra light chain LC3 confirm the conclusions previously drawn by Bhan et al. (1978) from comparative studies on the myosin isolated from hearts of normal and myopathic hamsters. LC3 in fact results from in vitro proteolysis of the light chain LC2. These authors, however, described a difference in the LC3 content between cardiac myosin from normal and pathological animals, which was attributed to different levels of neutral protease activity in the hearts of the latter. In contrast, the same level of in the sequence of 70% of the total LC1 and 50% of proteolytic activity was observed in normal and hypertrophied human hearts (Klotz et al., 1975). These results also make improbable the hypothesis of a possible relationship between the origin of LC3 and a heart disease (Henri and Sobel, 1973; Oganes-sian et al., 1973; Siemankowski and Dreizen, 1978). The sequence identity of LC3 with LC2 and its electrophoretic migration on two-dimensional gels is rather incompatible with the known properties of the fetal-specific form of cardiac light chains (Whalen et al., 1978; Cummins et al., 1980).

The absence of differences between the light chains from normal and hypertrophied human hearts suggests that the molecular basis for the differences in the properties of the myosin lies only in the heavy chains. There appear to be two possibilities, the first that one or more of the side chains are modified, and the second that a new gene is activated, leading to the production of a new heavy chain. The latter would be analogous to the situation in skeletal muscle. Following surgical reinnervation, fast muscles can be induced to synthesize myosin that is characteristic of slow muscle, and vice versa. In those studies, the light and heavy chains always appear to be matched, i.e., the synthesis of fast light chains is accompanied by the synthesis of fast type heavy chains, while slow light chains and slow heavy chains also appear synchronously (Buller and Mommaerts, 1969; Barany and Close, 1971). In abnormal hearts, if new heavy chains are made together with “normal” light chains, this would mean that two different types of heavy chain are combined, under normal vs. stress conditions, with the same light chains. Thyrotoxic myosin may also result from the combination of the “normal” light chain with “new” heavy chains (Flink et al., 1979).

There remains the possibility that side chain modification takes place in the heavy chain. Variation in the degree of methylation is a possibility, but “thyrotoxic” myosin (which shows an elevated ATPase activity) has been shown to be normal in
this respect (Barnejee et al., 1976). There are other possibilities, such as phosphorylation and glycosylation, and it remains to be seen whether these occur in myosin from abnormal hearts.

The results presented here demonstrate that the light chains of myosin from normal and hypertrophied human hearts are identical throughout much of their sequences, and therefore our tentative conclusion is that the light chains give no clues regarding the molecular basis for the observed enzymatic differences. Of course an unequivocal demonstration of complete identity of the light chains will require complete sequences analyses, and these have been hampered by lack of sufficient material. Analogous studies on the corresponding heavy chains may eventually tell us what factors are indeed responsible for changes in the enzymatic properties of the myosin synthesized after the heart is subjected to stress.

References
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