hydrophobic group associations and rupture of hydrogen-bonded conformations. It is therefore desirable to consider other criteria of denaturation that could be primarily hydrophobically motivated.

I would advocate the use of two additional criteria, namely increased pI values and lowered surface-tension values. Elsewhere (Lewin, 1972a) I have computed that de-adherence of hydrophobic associations (which lead to protein denaturation) should result in two phenomena, namely (a) increased pK values of constituent weak acid groups and (b) lowered surface-tension values.

(a) *Increased pK values of constituent weak acid groups.* This phenomenon would arise from the increased hydrophobic-group surface area, which in turn should result in corresponding decreased entropy (or higher organization) of the water molecules in contact with the newly liberated hydrophobic surface, and therefore in their decreased availability to participate in the ionization:

This effect results in corresponding increased relevant pK values and associated pI values. Experimentally it has already been demonstrated that the pK values of carboxylic acids rise on denaturation (see, e.g., Bailey, 1942); and electrofocusing carried out in this laboratory (Lewin, 1972a) recorded pI increases as high as 0.2–0.4 unit in a number of protein denaturations (caused by heat or urea), including those of serum albumin and haemoglobin (see also Salaman & Williamson, 1971).

(b) *Lowered surface-tension values.* This phenomenon would also arise from increased hydrophobic surface area, which, in accord with Traube's Rule, results in lowered surface-tension values. Elsewhere (Lewin, 1972a) this was computed to arise from specific restructuring of the water molecules' associations. Experimentally it was concluded (Lewin, 1972a,b) that protein molecules or their assemblies, which can undergo irreversible denaturation [e.g. low-density lipoproteins, high-density lipoproteins and human sera, which on freezing and thawing are known to undergo irreversible denaturation (see, e.g., Greaves, 1965; Davies, 1965; Lovelock, 1957; Oncley *et al.*, 1957; Scanu, 1965)], display a decrease in surface-tension value that can amount to as much as 2.5 dyn/cm from the normal value of 47 dyn/cm in the native state (see, e.g., Lewin, 1971).

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A Phosphorylated Light-Chain Component of Myosin

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The light-chain fraction of myosin prepared from the white skeletal muscle of adult rabbits under certain conditions can be resolved into four components by polyacrylamide-gel electrophoresis in 8M-urea in 20mM-tris-glycine buffer, pH 8.6 (Perrie & Perry, 1970). The two components of intermediate mobility MI_2 and MI_3 (for nomenclature see Perrie & Perry, 1970) migrated as a single band corresponding to a molecular weight of 18 500 on electrophoresis in the presence of sodium dodecyl sulphate and corresponded to the light-chain fraction removed from myosin by treatment with 5,5'-dithiobis-(2-nitrobenzoic acid) (Weeds, 1969; Gazith *et al.*, 1970). The molecular weights of the MI_1 and MI_4 components were estimated to be 15 500 and 22 000 respectively.

When myosin was extracted from muscle by 0.6M-KCl or by 0.3M-KCl in 0.15M-sodium phosphate buffer, pH 6.5, component MI_2 was either absent or present in only trace amounts. Its amount increased at the expense of component MI_3 by the inclusion of 10mM-sodium pyrophosphate, 10mM-ATP or 0.1M-NaF in the extraction buffer.

Amino acid analyses components of MI_2 and MI_3 isolated by a modification of the method of Perrie & Perry (1970) showed no significant differences and were in reasonable agreement with the analytical data of Weeds & Lowey (1971) for the 5,5'-dithiobis-(2-nitrobenzoic acid)-treated light chains. Gel electrophoresis of the CNBr-treated MI_2 and MI_3 components gave an essentially identical band pattern. Total phosphate determinations showed the presence of 1 mol of phosphate/mol of component MI_2 with no significant amounts in component MI_3 . The u.v.-absorption spectra of the two components were identical and tests for the presence of ribose were negative. Component MI_2 was converted into component MI_3 by the action of a pure alkaline phosphatase from *Escherichia coli*. When myosin was isolated in the presence of [γ - ^{32}P]ATP and 0.1M-NaF a small but significant amount of ^{32}P was

detected in component M_{12} but none in components M_{11} , M_{13} and M_{14} .

It is concluded that the light-chain component of molecular weight 18500 present in skeletal myosin can exist in either a phosphorylated or a non-phosphorylated form. The evidence also suggests that enzymic mechanisms exist for the phosphorylation and dephosphorylation of this light chain.

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A Proteolytic Fragment of 26000 Molecular Weight from the Heavy-Chain Fraction of Myosin

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Subfragment 1 prepared by digestion of rabbit skeletal-muscle myosin by the method of Lowey *et al.* (1969) with high papain/myosin ratios (1:200, by wt.) migrated as two components on polyacrylamide-gel electrophoresis in 40% glycerol in 20mM-tris-glycine buffer, pH8.6. The two components were present in roughly similar amounts. Subfragment 1 prepared by using a papain/myosin ratio 1:2000 contained only a single component with the same electrophoretic mobility as the slower component obtained by digestion with the higher papain/myosin ratio. In each case the band patterns obtained were identical whether the gels were stained for protein or for adenosine triphosphatase activity (Wachstein & Meisel, 1957).

On electrophoresis in 8M-urea in 20mM-tris-glycine buffer, pH8.6, the light-chain pattern of three components (Perrie *et al.*, 1969) was similar in both preparations. The heavy-chain fraction of subfragment 1 was studied by electrophoresis in sodium dodecyl sulphate (Weber & Osborn, 1969). Under these conditions components with molecular weights 15500, 18500 and 23000, which corresponded to the three light-chain components noted on electrophoresis in 8M-urea, could be detected in both preparations. In addition, the digest with the low papain/myosin ratio contained components with molecular weights 87000, 69000 and 26000 derived from the heavy-chain fraction. The digest with the high papain/myosin ratio, however, contained little or none of the '87000 component' but contained a '53000 component' together with the '69000' and '26000 components'. Densitometric traces indicated

that the amount of '26000 component' was higher in preparations that possessed more of the '53000 component'.

The '26000 component' isolated by gel filtration on Sephadex G-200 in sodium dodecyl sulphate migrated as a single band on electrophoresis in sodium dodecyl sulphate. It has an amino acid composition similar to that of the heavy-chain fraction of myosin and contained 0.6mol of mono- N^{ϵ} -methyl-lysine, 0.9mol of tris- N^{ϵ} -methyl-lysine and 0.3mol of 3(*tele*)-methylhistidine/mol of fragment (cf. Hardy *et al.*, 1970).

Thus it appears that, depending on the papain concentration, two species of subfragment 1 molecules are produced that differ mainly in the proteolytic cleavages, present in the heavy chains. It is of interest that, despite these cleavages in the heavy chains, both preparations possess adenosine triphosphatase activity.

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Subunit Structure and Biological Activity of Tropomyosin B from Different Muscle Types

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Tropomyosin is required in addition to the troponin complex to restore Ca^{2+} -sensitivity to the Mg^{2+} -stimulated adenosine triphosphatase of desensitized actomyosin (Ebashi & Kodama, 1966). It also acts independently as an inhibitor of the Ca^{2+} -stimulated adenosine triphosphatase of desensitized actomyosin (Schaub *et al.*, 1967). Whereas the former property is not affected by the blocking of the thiol groups of tropomyosin with iodoacetate, phenylmercuric acetate or bis-2-carboxyethyl disulphide, the latter property was lost after treatment with these reagents. Under defined conditions of pH and ionic strength heating in the range 55–60°C was more effective in destroying the activity of tropomyosin in the relaxing protein system than its inhibitory activity on the Ca^{2+} -stimulated adenosine triphosphatase. Elsewhere we have reported that the former property is more sensitive to controlled mild proteolysis than is the latter (Schaub & Perry, 1969).

In polyacrylamide-gel isoelectric focusing and