(b) Three bands, namely an origin band and two bands migrating towards the anode: Triton X-100 had the same effect on the origin band as in (a) but did not seem to affect the other two bands. (c) Two bands, namely an origin band and one anodic band: again Triton X-100 had a similar effect to that in (a) and (b). The bands were completely abolished by 10mM-NaF whether α -naphthyl phosphate or 4-methylumbelliferyl phosphate was used as substrate. Blood content of the tissues did not contribute significantly to the acid phosphatase pattern or activity.

Preliminary evidence suggests that extracts having a relatively high L(+)-tartrate inhibition (45–50%) also had strong anodic bands (i.e. those not mobilized by Triton X-100) that were completely abolished by this inhibitor. Electrophoresis of the 105000g pellet and supernatant suggested that the fastest anodic band was cytoplasmic rather than lysosomal or microsomal.

It is concluded that human endometrium may contain up to three isoenzymes of acid phosphatase. Work is in progress to relate the isoenzyme pattern and total activity with physiological and clinical states.

We are grateful to the Medical Research Council for a Scholarship for Training in Research Methods (to N. A. S.).

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The Order of Addition of Substrates to Yeast Hexokinase

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There is much controversy whether the addition of substrates to hexokinase is ordered or random, a very recent paper concluding that it is the latter (Rudolph & Fromm, 1971). We have therefore investigated this aspect of the reaction by flux techniques (Britton, 1966). This approach leads to independent tests that may be interpreted relatively unequivocally.

The ratio (flux of material from glucose 6-phosphate to ATP)/(flux from glucose 6-phosphate to glucose) when the ATP and glucose concentrations are altered in turn should vary characteristically according to the way in which ATP and glucose add to the enzyme. The ratio will not be affected by the glucose 6-phosphate concentration. Measurements were made at equilibrium, and therefore the con-

centrations of ATP and glucose 6-phosphate and those of glucose and glucose 6-phosphate were varied in pairs to keep the concentrations of the other substrates constant. In general for random addition the flux ratio plotted against ATP should yield a hyperbola with the ratio increasing from a value less than unity to a plateau greater than unity and a similar curve should be obtained when the reciprocal of the ratio is plotted against glucose. For ordered addition with glucose adding first the flux ratio should be independent of glucose concentration and should increase linearly from unity with ATP concentration. If part of the reaction should proceed via addition of ATP before glucose and if the step:

should be very fast the flux ratio will vary as described for ordered addition except that it will reach a maximum at high ATP concentrations. The contribution of the pathway can be calculated from this value.

Flux measurements were made with ^{32}P - and ^{14}C -labelled substrates at pH 6.5 in imidazole buffer; ionic strength (0.2) and the concentration of free Mg^{2+} (5.00mM) were kept constant. The flux ratio was independent of glucose concentration (1.50 ± 0.09 for 0.1–0.7mM-glucose), but it increased linearly from unity at zero ATP to 5 at 1.2mM-ATP. It is concluded that the addition of substrates to yeast hexokinase is essentially ordered. If any ATP should add first to the enzyme this pathway would seem to contribute considerably less than 10% to the overall reaction.

We thank the Science Research Council for financial support.

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Criteria of De-adherence of Hydrophobic Associations in Proteins

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Reversible and irreversible protein denaturations can take place during the processes of separation and isolation, and these can give rise to additional bands, sediments or peaks that could be mistaken for new compounds or 'artifacts'. Recognition of denatured states is therefore desirable, and certain physico-chemical properties such as hyperchromicity, lowering of optical rotation and corresponding changes in circular dichroism have been utilized in this connexion. These, however, can result primarily from changes that would involve both de-adherence of

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