(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.

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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.

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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