concluded that the appropriate activation energy is smaller than 0·05 k.cal./mole. These activation energies are much smaller than those measured by Arizumi and Kotani[5] and Kobayashi and Furaya[6] at higher pressures (5–8 k.cal./mole).

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Preparation of Dephosphorized Casein by an Enzymic Method

Plimmer and Bayliss[1] showed as early as in 1906 that the phosphorus of casein could be completely removed as inorganic phosphate by the action of 1 per cent sodium hydroxide at 37° C. in 24 hr. Acidification of the digest after the completion of dephosphorylation precipitated a substance resembling casein. Rimington[2] analysed the dephosphorized casein for its nitrogen distribution and noticed little difference in composition from that of casein except in amide and arginine nitrogen. The low values obtained in these two cases were evidently due to the hydrolytic action of alkali on these compounds with the production of ammonia. Plimmer and Lawton[3] found besides that by the action of $N/4$ alkali on casein there was formed in addition to casein a substance or mixture of substances resembling a primary proteose. The use of alkali as a dephosphorylating agent was considered to be undesirable for these reasons.

Enzymic methods have come into vogue recently in studies of this type. The preparation of dephosphorized phosvitin by Mecham and Olcott[4] and of phosphorus-free ovalbumin by Perlmann[5] deserves special mention in this connexion. In the present investigation a purified preparation of phosphoprotein phosphatase obtained by us from ox spleen[6] has been used for the preparation of dephosphorized casein. For the preparation of the protein the following method has been adopted.

1 litre of the digest containing 25 gm. of casein, 25 ml. of the pure enzyme preparation (283·5 phosphatase units per mgm. of protein nitrogen) and thioglycollic acid (activator) to a final concentration of 0·001 M was adjusted to a pH of 6·0 by veronal–acetate buffer. At the end of 24 hr. incubation at 37° C., a further quantity of enzyme preparation (10 ml.) was added to the digest. A precipitate had begun to separate by this time. When there was no more increase in the liberation of inorganic phosphorus (96 hr.) the precipitated protein was separated on the centrifuge, washed repeatedly with water and finally with acetone. The air-dry product weighed 16·5 gm.

The protein is a white amorphous powder lighter than casein. It is relatively insoluble in water. It is, however, soluble in dilute alkali and its solubility in dilute acids is greater than that of casein. It can be precipitated from its alkali solution by acidifying to pH 6·0. There seems to be thus a shift in the isoelectric point of casein to the alkaline side during its dephosphorylation, presumably due to the removal of the phosphoric acid groups.

The protein has a nitrogen content of 15·7 per cent and contains about 0·03 per cent of phosphorus. Its content of arginine, histidine, cystine and tryptophan as determined by standard chemical methods resembles closely that of casein. No drastic changes appear to take place during enzymic dephosphorylation. The protein has also been found to be readily attacked by proteolytic enzymes. Alkali-dephosphorylated casein is attacked by these enzymes with difficulty[2], possibly due to some racemization having taken place during alkali treatment. The lowering in nitrogen content brought about by alkali is another serious defect in the use of this reagent as a dephosphorylating agent. Since these drawbacks are eliminated by enzymic dephosphorylation, phosphoprotein phosphatase should prove to be a useful tool in studies on dephosphorylated proteins.

The complete amino-acid make-up of the protein is under investigation, and details of this work will be published elsewhere.

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"Role of Plasmagenes"

IN Prof. C. H. Waddington's review of the book by Boris Ephrussi, "Nucleo-cytoplasmic Relations in Micro-organisms"[1], the major adverse criticisms do not, in my opinion, stand up to close examination.

First is the imputation that Ephrussi does not understand, or at least recognize, that "A property of genetic continuity is attributed to both nuclear genes and plasmagenes, but the evidence for it is of quite different, in fact almost opposite, nature in the two cases. Nuclear genes are considered to exhibit genetic continuity because they continue in existence throughout lengthy series of crosses, etc., whereas the evidence for the continuity of plasmagenes is the fact that if they are got rid of they cannot be regenerated". Now the fact is that, whereas nuclear genes and plasmagenes have been detected on the basis of somewhat different criteria, their common attribute of genetic continuity is based on essentially the same properties and observations. In so far as present knowledge permits the definition of plasmagenes and nuclear genes, Waddington might have more soundly compared them along the following lines, which would have led to a quite different approach to the point at issue.

(1) Neither a nuclear gene nor a plasmagene is recognizable unless found in differential states of activity and inactivity, or presence and absence.

(2) Both are considered to exhibit genetic continuity because they continue in a lengthy series of cell generations over which some characteristic referring back to the 'gene' is faithfully recurrent.