In Fig. 2 are given diagrams of the same rabbit serum analysed simultaneously in the four different compartments. The boundaries at η are due to accumulation of protein and salts at the cellophane membrane. As would be expected the diagrams are almost exactly identical.

We are much indebted to Messrs. Hilger and Watts for making the glass parts of the cell and lending them to this unit.

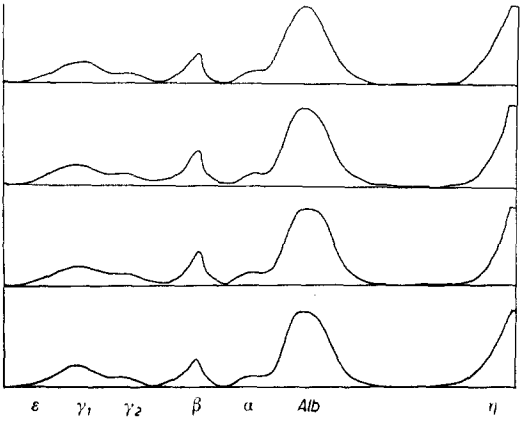

Fig. 2. Electrophoresis diagrams of the same rabbit serum analysed simultaneously in the four compartment cell. Electrophoresis time 150 minutes, Voltage gradient 5 volts/cm, Borate buffer pH 8.6.

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REFERENCES

[1] A. POLSON, *Nature*, 170 (1953) 628.
[2] A. POLSON, *Onderstepoort J.*, 20 (1945) 159.

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PHOSPHODIESTER LINKAGES IN PROTEINS

by

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As shown recently[1], prostate phosphatase liberates readily 40% of the phosphorus of α-casein but does not act on β-casein. This observation, together with the fact that prostate phosphatase in the pH range of 5.6 to 6.0 dephosphorylates monoesters of the type $-O-P\leqslant^{O}_{(OH)_2}$, indicates that the phosphorus in β-casein is present in a linkage different from that found in ovalbumin[2] and in α-casein[1]. As will be demonstrated below, β-casein contains the phosphorus in form of diesters.

The β-casein used in these experiments was kindly provided by Dr. THOMAS L. MCMEEKIN of the Eastern Regional Laboratories. The phosphodiesterase was prepared from rattle snake venom (*Crotalus adamanteus*) essentially according to SINSHEIMER[3]. These preparations contain as impurity 5-nucleotidase, an enzyme which does not act on proteins and therefore was not eliminated. However, care was taken that our preparations were free of proteolytic activity as tested with the aid of the hemoglobin method[4]. No proteolysis occurred at pH 5.6 and 8.2 during 24 hours.

A ten ml sample consisting of 100 mg β-casein in 0.05 *M* sodium bicarbonate was incubated at 37° with 10 γ diesterase. The reaction mixture contained 0.01 *M* magnesium ion. After six hours, a two ml sample was withdrawn for the estimation of phosphorus. At this point, no inorganic phosphorus was liberated. The pH of the solution, however, had shifted from pH 8.2 to 7.9, indicating the exposure of acidic groups. The remaining eight ml were then diluted to 30 ml with 0.06 *M* sodium acetate buffer of pH 5.3. To 15 ml of this solution was added 400 γ prostate phosphatase and the mixture incubated at 37° C. The second 15 ml portion, not containing the prostate enzyme, was used as control. A separate set of controls consisting of β-casein in 0.05 *M* sodium bicarbonate without added phosphodiesterase was treated in identical manner.

After incubation for 14 and 24 hours, respectively, the protein in a four ml aliquot was coagulated by the addition of an equal amount of 20% trichloroacetic acid and immersion of the mixture in a boiling water bath for five minutes. The inorganic phosphorus was then determined on the filtrate after removal of the precipitated protein by filtration. The results are summarized in Table I.

TABLE I

ACTION OF PROSTRATE PHOSPHATASE ON β-CASEIN PRETREATED WITH SNAKE VENOM DIESTERASE

Reaction mixture	*pH of reaction mixture*	*Time of incubation at 37° in hours*	*Phosphorus released by enzyme % of total phosphorus*
β-casein in 0.05 *M* $NaHCO_3$ + phosphodiesterase	8.2 → 7.9	6	0
β-casein, phosphodiesterase treated in acetate	5.8	24	0
β-casein, phosphodiesterase treated + prostate phosphatase	5.8	14	54
β-casein, phosphodiesterase treated + prostate phosphatase	5.8	24	72
β-casein in 0.05 *M* $NaHCO_3$, Control	8.2 → 8.2	6	0
β-casein (Control) + prostate phosphatase	5.8	24	0

From the experiments presented here, it is apparent that the phosphorus in β-casein is present in form of diesters —O—P—O—. Thus the rupture of one —O—P— bond by the diesterase exposes monoester groups which reduce the pH of the solution but do not lead to the appearance of inorganic phosphate which is liberated only if the prostate phosphatase is present to act on the monoesters. It was further noticed that after removal of the phosphorus trichloroacetic acid-soluble material, *i.e.* peptides, was present in the solution as detected both by the ninhydrin reaction and by absorption in the ultraviolet at 280 μm. Thus it is clear that the diester bonds in β-casein link peptide chains, some of which may be relatively short.

Although not shown in Table I, evidence has accumulated that the one atom of phosphorus of pepsin is also present in form of a diester. Pepsin, however, possesses only one N-terminal group[5,6] indicating that it is a protein with one peptide chain. The phosphorus therefore must crosslink a portion of the chain into a cyclic loop.

Previous investigators[7] have suggested phosphodiester bonds in phosphopeptones. The above mentioned results are, however, the first experimental demonstration of the existence of such linkages in proteins, thus adding a new type of crosslink to that of the disulfide bonds. As in the case of the sulfur bridge, the phosphorus in proteins can either link peptide chains, *e.g.* β-casein, or close a ring, *e.g.* pepsin.

REFERENCES

1 G. E. PERLMANN, *J. Am. Chem. Soc.*, 74 (1952) 3191.
2 G. E. PERLMANN, *J. Gen. Physiology*, 35 (1952) 711.
3 R. L. SINSHEIMER AND J. F. KOERNER, *J. Biol. Chem.*, 198 (1952) 293.
4 M. L. ANSON in NORTHROP, KUNITZ AND HERRIOTT, *Crystalline Enzymes*, Columbia University Press, New York, N.Y. 2nd edition, 1948, p. 305.
5 R. M. HERRIOTT, personal communication.
6 M. B. WILLIAMSON AND J. M. PASSMANN, *J. Biol. Chem.*, 199 (1952) 121.
C. RIMINGTON, *Biochem. J.*, 21 (1927) 1179;
cf. C. RIMINGTON AND H. D. KAY, *Biochem. J.*, 20 (1926) 777.

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