centrifuge showed that all the radioactivity was in the supernatant fraction. Further fractionation on Sephadex G-25 and G-75 showed two peaks, one of which had a high radioactivity count and a high E_{250} value. The presence of cadmium and thiol groups was confirmed. The protein resembles metallothionein in spectral and chromatographic properties. Attempts are under way to prepare this protein in quantity, to test the homogeneity of the preparation and to measure other physicochemical properties.

Kagi, J. H. R. (1970) *Abstr. 8th. Int. Congr. Biochem., Switzerland*, p. 130

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The Denatured State of Ovalbumin

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The denatured state of a protein obtained in 6M-guanidine hydrochloride plus 2-mercaptoethanol can be characterized by, among other things, intrinsic viscosity $[\eta]$. With increase in temperature bond rotation in the polypeptide chain will be facilitated, and therefore the chain will be shortened, which will result in a decrease in $[\eta]$. The denatured state therefore can be further characterized from measurements of $[\eta]$ as a function of temperature. A notable decrease in $[\eta]$ with temperature must be observed for the denatured protein. This diagnostic criterion of a denatured protein has been generally ignored. In the present communication we present our findings on the temperature-dependence of the intrinsic viscosity of the denatured state of ovalbumin.

The intrinsic viscosity of ovalbumin was measured in 6M-guanidine hydrochloride plus 2-mercaptoethanol at pH 4.85 and at different temperatures in a Cannon-Fenske viscometer with a flow time of 482.0 s for water at 30°C. The intrinsic viscosity of the completely unfolded ovalbumin was found to be 37.4 ml/g at 25°C, which is in agreement with the value calculated by the empirical equation of Tanford (1968). The viscosity showed little or no change in the temperature interval 25–35°C. However, as expected, at higher temperatures the intrinsic viscosity was considerably decreased, confirming our finding that in 6M-guanidine hydrochloride plus 2-mercaptoethanol ovalbumin exists as a randomly coiled protein.

Tanford, C. (1968) *Advan. Protein Chem.* **23**, 121

Studies on Ovomuroid: Hydrogen Ion Equilibrium in the Acid pH Range

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Ovomucoid is an important trypsin inhibitor and is known to interact with the enzyme. Although it is known, in a general way, that this protein-protein interaction prevents the expression of the biological activity of trypsin, very little is known about the mode and extent of this interaction. Information about the native conformation of ovomucoid and the nature of its accessible chemical groups should be helpful for a proper understanding of the trypsin-ovomuroid interaction. This prompted us to investigate the nature of the ionizable groups in ovomucoid. In the present communication we present our findings on the carboxyl groups of ovomucoid.

The H^+ ion titration curve of ovomucoid was obtained by using the cell assembly recommended by Tanford (1955). Ovomuroid was characterized on a DEAE-cellulose column. A symmetrical peak was obtained. The pI of the protein was found to be 4.4, which increased to 4.6 at I 0.15 and at 25°C. The protein concentration was 0.46–0.47%. From the H^+ ion titration curve of ovomucoid it was found that nearly 35 carboxyl groups titrate between pH 1.49 and 6.0, and the value of the electrostatic interaction factor, w , was found to be 0.033, which was similar to the calculated value ($w = 0.033$). The value of w remained unaltered throughout the pH range used in the titration. The electrostatic free energy of native ovomucoid was found to be 9.2 kJ/mol (2.2 kcal/mol) at pH 3.5.

Tanford, C. (1955) in *Electrochemistry in Biology and Medicine* (Shedlowsky, T., ed.), p. 248, John Wiley and Sons, New York

Structural Requirements of Inducer for the Formation of Penicillin Acylase in *Fusarium* sp. 7.5–5

By S. B. THADHANI, P. S. BORKAR and S. RAMACHANDRAN (*Antibiotics Research Centre, Pimpri, Poona-13, India*)

Penicillin acylase, an enzyme hydrolysing phenoxy-methyl-penicillin to 6-aminopenicillanate and phenoxyacetate, was purified 200-fold from the culture filtrate of *Fusarium* sp. and was homogeneous on polyacrylamide-disc-gel electrophoresis.

The enzyme was formed adaptatively when the organism was grown in the presence of phenoxyacetate or its derivatives. The amount of enzyme induced depended on the nature of the substitution

of phenoxyacetate and on the amino acid moiety of the phenoxyacetyl-L-amino acid. The acylase activity was highest with phenoxyacetyl-L-alanine and decreased with higher homologues of aliphatic amino acid. The presence of phenyl, heterocyclic or hydroxyl groups on the side chain or the absence of the α -carboxyl group in the amino acid moiety did not decrease the inducer effect of the compound. Phenoxyacetyl-D-alanine failed to induce the enzyme formation by the organism, indicating that the enzyme formation exhibited marked stereospecificity towards the amino acid moiety of phenoxyacetyl-amino acid. Although the phenoxyacetyl-amino acids served as good inducers of the enzyme, none of them was acted upon as a substrate by the enzyme.

Effect of Hydrogen Peroxide on Buffalo Milk Proteins

By A. SRINIVASAN (*Department of Chemistry, Loyola College, Madras-34, India*)

The use of H_2O_2 for the preservation of milk and in the production of special varieties of cheese has aroused interest in recent years. In tropical countries, because of the high atmospheric temperature and humidity and also because of the non-sterile conditions used for milking, fresh milk readily turns sour. The addition of small quantities of H_2O_2 (0.03–0.1 %, w/w) to fresh milk not only preserved the milk for over 36–48 h but also effectively controlled and diminished the bacterial count. The composition of the various constituents, particularly the proteins, and the flavour of milk were not affected by the addition of H_2O_2 . Moreover H_2O_2 was readily destroyed by the catalase activity, leaving no trace of it in the milk. Hence the use of H_2O_2 for the short-term preservation of milk assumes special significance in tropical countries.

Studies on the effects of different concentrations of H_2O_2 , over different time-intervals, on the composition of buffalo casein and whey proteins (Lakshminarayana & Dastur, 1968) have shown that at lower concentrations it acts as a short-term preservative, whereas at higher concentrations (0.2–0.5 %, w/w) it affects the flavour and the protein content and attacks the amino acids, particularly the sulphur-containing amino acids and tyrosine (Srinivasan, 1969).

Laxminarayana, H. & Dastur, N. N. (1968) *Dairy Sci. Abstr.* 30, 177–186

Srinivasan, A. (1969) Ph.D. Thesis, University of Madras

Comparative Study of the Thyroid-Gland and Submaxillary-Gland Peroxidases

By INDIRA HALDAR, USHA MAHAJANI and ASOKE G. DATTA (*Indian Institute of Experimental Medicine, Jadavpur, Calcutta-32, India*)

A peroxidase (EC 1.11.1.7) was isolated from the soluble supernatant of the thyroid gland of goat and purified to homogeneity as ascertained by immunodiffusion, chromatography, ultracentrifugation and polyacrylamide-gel electrophoresis. General properties of the enzyme were studied and the prosthetic group was characterized as ferric protoporphyrin IX. Many of the molecular properties of this enzyme are similar to those of the goat submaxillary-gland peroxidase purified previously. Both the thyroid-gland and the submaxillary-gland peroxidase are found to be serologically similar and do not catalyse an iodination reaction. Their behaviour towards some common inhibitors and their molecular weights are comparable, but they show slight differences in their catalytic properties. However, there is a distinct difference in the electrophoretic mobilities of the two enzymes. The amino acid compositions of the two enzymes were also compared. The electrophoretic, chromatographic, enzymological and immunological properties of goat thyroid-gland and submaxillary-gland peroxidases suggest that peroxidases exist in multiple molecular forms not only in plants but in mammalian systems also.

Effect of α -p-Chlorophenoxyisobutyrate on Oxidative Phosphorylation

By S. R. PANINI (*Department of Biochemistry, Indian Institute of Science, Bangalore-12, India*)

The effect of the antihypercholesterolaemic drug ethyl α -p-chlorophenoxyisobutyrate (clofibrate, Atromid-S) on oxidative phosphorylation by rat liver mitochondria was studied. The sodium salt of the free acid inhibited State 3 oxidation with NAD^+ -linked substrate (glutamate+malate) as well as with succinate. The concentrations for 50% inhibition were 2.2 and 3.5 mM with glutamate+malate and succinate respectively. The respiratory-control index decreased with increasing concentrations of the drug. The concentration required for a 50% decrease in respiratory control was 1.0 mM with both glutamate+malate and succinate. The inhibition exerted by the drug on State 3 oxidation was released by 2,4-dinitrophenol. However, at high concentrations of the drug the reversal was either partial or negligible. High concentrations of the drug stimulated O_2 uptake with ascorbate+cytochrome *c* as substrate. The concentration of the drug required for 50% stimulation was