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).

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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

9.3 mM. The ADP/O ratio (glutamate+malate) was decreased by 1 at drug concentrations above 2.5–3.0 mM. These concentrations had no effect on the ADP/O ratio with succinate as substrate.

Acyl Phosphatase of *Vigna catjang*

By V. K. RANJEKAR and V. JAGANNATHAN (*National Chemical Laboratory, Poona-8, India*)

Acyl phosphatase has been obtained in highly purified form from brain (Diederich & Grisolia, 1969), muscle (Ramponi *et al.*, 1969; Shiokawa & Noda, 1970) and erythrocytes (Rakitzis & Mills, 1969) of animal tissues, but the enzyme from plants has not been studied hitherto. Four different enzymes that hydrolyse acetyl phosphate have been shown to be present in seeds of *Vigna catjang*; only one of these is specific for acetyl phosphate and does not hydrolyse other phosphate esters. This enzyme was purified about 400-fold and obtained in electrophoretically homogeneous form. It has a sedimentation coefficient of 1.2S and resembles the enzyme from animal tissues in its low molecular weight, inhibition by P_i and stability at low pH. However, it differs from the animal enzyme in being inhibited irreversibly by *p*-chloromercuribenzoate and Hg^{2+} and reversibly by bisulphite. It is inactive towards carbamoyl phosphate. It has no metal ion requirement and is stable towards di-isopropyl phosphorofluoridate. Its properties and kinetics, its variation in plant tissues under different conditions and its possible metabolic role will be discussed.

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Ramponi, G., Guerritore, A., Treves, C., Nassi, P. & Baccari, V. (1969) *Arch. Biochem. Biophys.* **130**, 362

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Studies on Phosphatases of the Mosquito *Culex pipiens fatigans*

By M. B. LAKSHMI and D. SUBRAHMANYAM (*Department of Biochemistry, Postgraduate Institute of Medical Education and Research, Chandigarh, India*)

The alkaline phosphatases of the mosquito *Culex pipiens fatigans* were separated into two molecular species by chromatography on DEAE-Sephadex and their properties were studied. Zn^{2+} is essential for activity.

The insects also contain different acid phosphatases having pH optima 3.4 and 4.8 respectively with β -

glycerophosphate and with *p*-nitrophenyl phosphate as substrate. The enzyme with pH optimum 4.8 is also active on 5'-nucleotides.

The alkaline phosphatase of the insects readily hydrolyses the lipid phosphate esters, whereas the acid phosphatase is inactive. The distribution of the phosphatases in subcellular fractions and in different developmental stages of the insects and their characterization will be discussed.

Purification and Properties of Arylsulphatase A from Chicken Brain

By A. A. FAROOQUI and B. K. BACHHAWAT (*Neurochemistry Laboratory, Department of Neurological Sciences, Christian Medical College and Hospital, Vellore-4, India*)

Lysosomal arylsulphatases A and B are widespread in their distribution and have been found in all animal species studied, but very little is known about the role of these hydrolases in animal tissue. A significant decrease in arylsulphatase A activity in the genetic disorder metachromatic leucodystrophy, which is characterized by abnormal accumulation of sulphatides, has been observed in our laboratory (Austin *et al.*, 1963). Mehl & Jatzkewitz (1968) have reported that arylsulphatase A from pig kidney, in the presence of a heat-stable polymer, can degrade cerebroside 3-sulphate.

Previous work from this laboratory has indicated the differences in the proportions and physico-chemical properties of brain arylsulphatases in various animal species (Farooqui & Bachhawat, 1971). Chicken brain arylsulphatase in some respects resembles arylsulphatase A of other animal species in its kinetic properties such as K_m value, anomalous time-activity relationship and inhibitory effects of phosphate and sulphite ions. On the other hand its electrophoretic mobility and behaviour during zinc acetate fractionation are similar to those of arylsulphatase B of other species. Because of these properties, we undertook the purification of this enzyme from chicken brain. The enzyme was purified 2000-fold by using ammonium sulphate fractionation, ethanol precipitation, Sephadex-gel filtration and DEAE-Sephadex column chromatography. The purified enzyme is markedly inhibited by multivalent anions such as sulphite, phosphate and sulphate; the lowest degree of inhibition was given by sulphate. Univalent anions such as chloride have no effect, whereas fluoride ions markedly inhibit the enzyme activity. Like ox liver arylsulphatase A (Roy, 1970), this enzyme is inhibited by certain carbonyl reagents and ascorbic acid in the presence of metal ions. Cu^{2+} seems to be the ion