still involve the pre-viral nucleic acid molecule and the data of hydroxylamine-treatment appeared to suggest that the action of this amine is to break appropriately chromosomal nucleic acid to liberate the pre-virus. The cellular deoxyribonucleic acid was therefore mixed with 0·005 M hydroxylamine and after 2 h at 35° C the mixture was centrifuged with a 'Spinco *L*' ultracentrifuge according to the procedure mentioned above. It was corroborated that the yield of 35 *S* amounts to 6 per cent of the cellular nucleic acid employed. The centrifugation of the intact cellular deoxyribonucleic acid which had not been treated with a blender or hydroxylamine gave only about 2 per cent 35 *S* component. It can thus be confirmed that the pre-virus is pro-existent in the larval cell and that its separation is promoted by suitable means.

Recently, Northrop[7] demonstrated that a new phage can be produced by a long treatment of *Escherichia coli* with triethylene melamine. Ikuta and Zimmerman[8] also proved that a new virus is produced in the precancerous state of mice implanted with carcinogenic hydrocarbons. These results may be explained by the appropriate cleavage of cellular nucleic acids by chemicals. It is further assumed that duplicants proposed by Butenandt[9], Druckrey[10] and Euler[11] for precursors of cancers may correspond to pre-viral nucleic acids.

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Pyridoxal and Pyridoxamine Phosphate Breakdown by an Acid Phosphatase Preparation

DURING investigations of the controlled inactivation of pyridoxal phosphate-dependent enzymes, an acid phosphatase preparation has been used to effect co-enzyme destruction. Alkaline phosphatases of mammalian origin are known to hydrolyse both pyridoxal and pyridoxamine phosphates[1,2] and an alkaline phosphatase preparation from *E. coli* has been used for the resolution of tryptophanase[3]. A preliminary report on the inactivation of L-α-alanine : phenylglyoxal transaminase by treatment with alkaline phosphatase has also appeared[4]. The breakdown of vitamin B_6 phosphates by acid phosphatases does not appear to have previously been reported.

In the present work a commercial preparation of wheat germ acid phosphatase (B.D.H., Ltd.) was used. Activities with a variety of substrates were measured by the colorimetric assay of orthophosphate production according to the method of Gomori[5], readings being made at 715 mμ. Reaction mixtures contained buffer (0·2 M), 1·0 ml.; enzyme preparation in water 0·4 ml., substrate (7·0 mM), 0·5 ml.; and additions up to 3·5 ml. Incubations were carried out at 37° C and terminated by the addition of 0·5 ml. trichloroacetic acid (50 per cent).

With pyridoxal phosphate as substrate, preliminary experiments showed that optimum activity occurred at *p*H 5·5. Acetate buffers were used in this region, maleate and veronal buffers being used to cover a wider range of *p*H. Pyridoxal phosphatase activity rapidly declined between *p*H 5·5 and 7·0, and no evidence of a second peak at the alkaline side of neutrality was obtained, even at high enzyme concentrations. Pyridoxamine phosphate was hydrolysed optimally at the same *p*H but at a much lower rate. The respective specific activities were 88·0 and 10·8 mU. per mg protein, compared with 340·0 mU. per mg for disodium phenylphosphate used as a reference substrate.

Magnesium ions, as magnesium sulphate, had no effect on pyridoxal phosphatase activity at a concentration of 1 mM, and EDTA had no inhibitory effect at concentrations below 0·1 mM although 30 per cent inhibition was observed at 1 mM. Sodium fluoride caused marked inhibition at concentrations as low as 10·0 μM (10 per cent) and inhibited 70 per cent at 1 mM. Iodoacetate, β-mercaptoethanol, or cysteine had no effect on phosphatase activity when tested over the same concentration range, after 30 min preincubation with enzyme. Preincubation of pyridoxal phosphate in buffer with cysteine or histidine, which are known to form respectively thiozolidine and imidazo-tetrahydro-pyridine derivatives with the coenzyme[6], had no detectable effect on the rate of enzymatic hydrolysis.

The effect of substrate concentration on the initial rates of enzyme activity, using either pyridoxal or pyridoxamine phosphates, was investigated. Michaelis constants for these substrates were determined from double-reciprocal plots by the method of Lineweaver and Burk[7], and found to be 0·52 and 1·30 mM, respectively, when measured at *p*H 5·5.

The foregoing results show that wheat germ acid phosphatase readily hydrolysed pyridoxal phosphate. Pyridoxamine phosphatase activity was lower, however, and less favoured by the Michaelis constant. This result is in contrast to the results obtained using mammalian preparations[1,2]. It is interesting to note that an alkaline phosphatase from human brain hydrolysed pyridoxal phosphate more rapidly than all other substrates tested[2], and that whereas a bacterial acid phosphatase preparation had no activity with vitamin B_6 phosphates[3], a preparation of prostatic acid phosphatase was also more effective with pyridoxal phosphate than any other substrate[8]. The possible role of phosphatases in the regulation of pyridoxal phosphate requiring enzymes has been discussed elsewhere[9,10].

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Loss of Adenine during the Hydrazine Degradation of Deoxyribonucleic Acid

THE treatment of deoxyribonucleic acid (DNA) with anhydrous hydrazine causes the degradation and removal of the pyrimidine bases but leaves the rest of the polynucleotide chain more or less intact[1,2]. This degradation of the pyrimidine ring occurs irrespective of whether the pyrimidines occur as part of a nucleic acid or as free base, nucleoside or nucleotide[1–5]. This reaction has recently been considered in detail by Temperli *et al.*[5]. The pyrimidine-free DNA (apyrimidinic acid) can be hydrolysed by potassium hydroxide[4] to tracts of oligopurine nucleotides having the general formula, $(\text{purine nucleoside})_n$ $(\text{phosphate})_{n+1}$, where n has been observed to vary from 1 to 13