studied the kinetics of hydrolysis in solvent water in which, because of the high ionizing capacity of the medium, an S_N2 pathway is even less likely Our results (Table I) show that non-common ion

TABLE I

Effect of added ions on the rate of hydrolysis of 1-phenylneopentyl chloride in water

Temp. 45·70° C (RC1) = 0·0006 M

Added salt	Concentration (M)	$10^4 k_1$ (s^{-1})	% increase in rate due to added salt
Nil	..	1·47	..
$LiClO_4$	0·05	1·47	0·0
$NaNO_3$	0·05	1·52	2·7
NaBr	0·01	1·63	10·9
,,	0·05	2·04	38·8
,,	0·10	2·15	46·3

electrolytes show negligible ionic strength effect, which is consistent with the Debye–Hückel theory. Addition of bromide ions on the other hand shows marked increase in rate; the non-linearity between the increase in rate and the bromide ion concentration clearly indicates that its effect is not due to the normal type of salt effect described by Winstein[8]. Further support for this conclusion comes from the observation that the rate constants in the presence of bromide ions increase with the progress of reaction. This effect is not seen either in the absence of bromide ions or in the presence of other non-common ion electrolytes.

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THE OBSERVED ACTIVATION OF ENZYMES BY Mn^{++} IS SOMETIMES AN ARTIFACT

DURING a study of the effect of metal ions on the activity of a bacterial malic dehydrogenase (EC 1.1.1.37), it was found that Mn^{++} at concentrations as low as 10^{-5} M stimulated the rate at which absorption at 340 nm increased when malate and NAD were in excess. When oxaloacetate (OAA) and NADH were in excess, addition of Mn^{++} reduced the rate at which absorption at 340 nm decreased (Fig. 1). The kinetics of these reactions suggested the presence of a contaminating activity such as malate-NAD-oxidoreductase (EC 1.1.1.38). This enzyme, which is activated by Mn^{++}, catalyzes the conversion of malate to pyruvate and carbon dioxide[1].

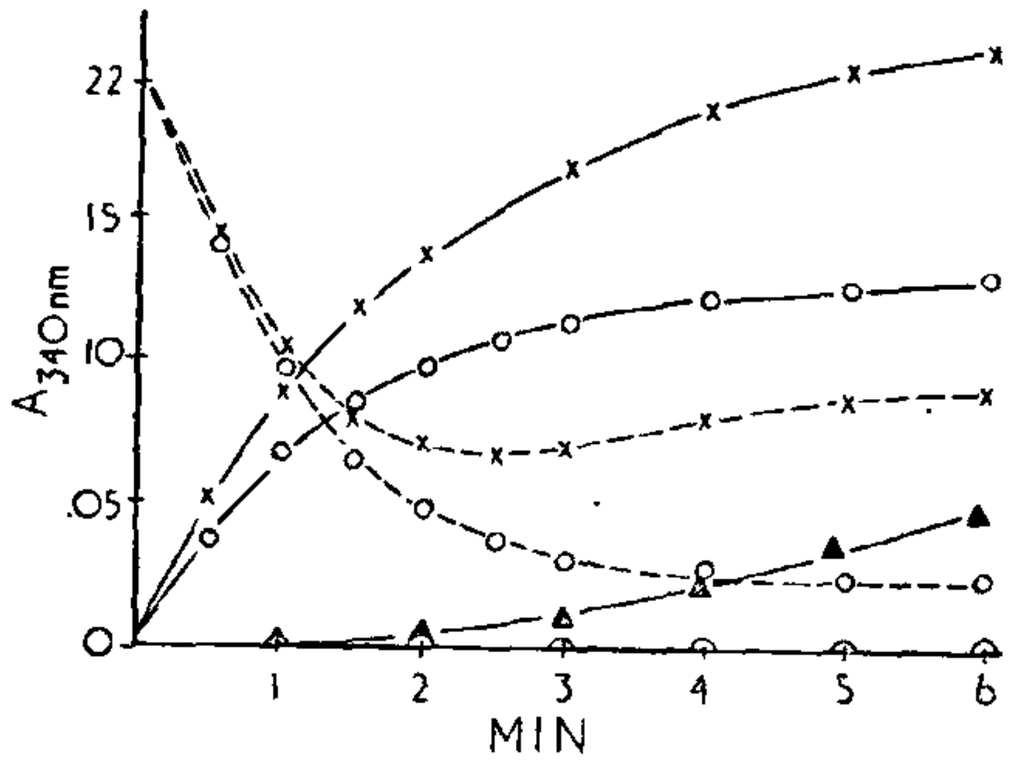

FIG. 1. Effect of Mn^{++} on a malic dehydrogenase (EC 1.1.1.37). Assays were done spectrophotometrically at 340 nm. Dotted lines: enzyme with oxaloacetate and NADH in tris buffer at pH 8·5. Solid line with circular symbols: enzyme with malate and NAD at pH 8·5. Curves with X symbols contained in addition 1 mM Mn^{++}. Curve with triangles: buffer only *plus* 1 mM Mn^{++} at pH 8·5 Base line (half circles): buffer at pH 7·0 *plus* 1 mM Mn^{++}, or buffer at pH 8·5 with 1 mM EDTA *plus* 1 mM Mn^{++}, or buffer alone.

We sought direct evidence for the presence of this enzyme by testing for the formation of both pyruvate and CO_2, the latter was determined manometrically after acidification. The CO_2 resulted from the spontaneous decomposition of OAA and its amount was independent of the Mn^{++} concentration. What pyruvate was detected by gas-liquid chromatography (GLC) was also unrelated to metal ions and was traced, in part, to the breakdown of OAA during methylation for GLC. Pyruvate was also estimated with lactic dehydrogenase; no significant increase could be attributed to the presence of Mn^{++} in the malic dehydrogenase assay. Further protein purification

steps did not increase the specific activity of the Mn-dependent activity, nor did it alter the fact that Mn^{++} stimulated the rate of reaction in one direction but not in the other. This suggested a non-enzymatic reaction. Careful examination of the system showed that the absorbancy of solutions of Mn^{++} in buffer at pH 8·5 increased steadily with time at a rate of the order of that expected for enzymic preparations. This effect was obviated by pH values below 7·0 or in the presence of ethylenediaminetetracetic acid (EDTA) as shown in Fig. 1. The rate of change in absorption was dependent on the pH and also the presence of dissolved oxygen. The reaction is, in fact, the oxidation of Mn^{++} to Mn^{+4}. The product, which is probably largely $Mn(OH)_4$ or a mixture of basic salts, is soluble at low concentrations. The solutes become colloidal and finally precipitate particularly at higher initial Mn^{++} concentrations and at higher pH values. A fairly large number of enzymes are activated by Mn^{++}. We believe, however, that some reports of stimulation by Mn^{++} (but not by other metal ions) should be re-examined. For example the NAD-linked glucose dehydrogenase of *Bacillus cereus* is reported activated at pH 8 by 10^{-4} M Mn^{++}. Other metals had no effect. Stimulation by Mn^{++} is demonstrated, but the term—activation—which is used *may* be misleading[2]. Was the appropriate control (Mn^{++} in buffer alone) run? It is extremely important that it should be particularly with spectrophotometric assays at wavelengths below about 450 nm at pH 7 or above, in the presence of air.

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CYCLIC NUCLEOTIDE PHOSPHODIESTERASE ACTIVITY IN *CICER ARIETINUM*

CYCLIC 3 : 5 adenosine monophosphate (cAMP) mimics the action of indol-yl-3-acetic acid (IAA) in stimulating the activity of tryptophan oxygenase in *Cicer arietinum*[1]. Furthermore, exposure of seedlings to IAA leads to an increase in the adenyl cyclase activity[2]. cAMP also stimulates RNA and protein synthesis[3] and protein phosphorylation[4] in the seedlings. These findings suggest a regulatory role for cAMP in germination[5] and it was of interest, therefore, to examine the activity of cAMP phosphodiesterase which mediates the hydrolysis and thereby controls the intracellular concentration of cAMP in living cells. cAMP phosphodiesterase activity[6] was present in dormant seeds and registered a significant rise after 72 hr germination. The enzyme prepared from 72 hr seedlings (whole seeds *miuns* testae and cotyledon) was localized in the supernatant fraction recovered after sedimenting organelles at 100,000 g. The enzyme had an optimum pH around 5 and hydrolysed besides cAMP, cyclic uridine monophosphate, cyclic guanosine monophosphate and dibutyryl cAMP. Theophylline or theobromine in a concentration range of 100 μM–1 mM had no inhibitory effect and imidazole (100 μM) or IAA up to 1 mM had no activating effect on the enzyme. The plant enzyme thus seems to be different from mammalian cyclic AMP phosphodiesterases.

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OCCURRENCE OF AVICULARIN IN THE LEAVES OF *CINCHONA OFFICINALIS* AND *C. ROBUSTA*

THE genus *Cinchona* belonging to the family Rubiaceae and well-known for its alkaloids, has not been studied in any detail for the presence of polyphenols. We have earlier reported[1] the occurrence of mannitol in twenty plants and flavonoids in seven members of this family. The isolation of reynoutrin (a compound mis-identified earlier as a xyloside[2] and later shown to be an arabinoside[3]) from *Cinchona ledgeriana*[4] has been reported. In view of these it was considered desirable to examine the leaves of