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

C. officinalis and *C. robusta* grown in South India and our findings are recorded below.

Adopting the standard procedure for the isolation of flavonoids, the aglycones present in the leaves were found to be mainly quercetin with small quantities of kaempferol. Of the three glycosides present, the one in appreciable quantity was isolated in pure form as light yellow needles, m.p. 210–12° (decomp). It was purple under U.V. changing to yellow with NH_3 and had λ_{max} (nm) 259, 359 and λ_{min} 238, 286 (EtOH) and showed shifts with diagnostic reagents[5] characteristic for a quercetin-3–0–glycoside. Its IR spectrum (KBr) exhibited bands at 1650, 1600, 1500, 1360, 1195, 1110, 1040, 1000, 945, 920; 825 and 800 cm^{-1}. On acid hydrolysis it yielded quercetin and L–arabinose in equal proportion. The identity of the sugar as L–arabinose was established by co-chromatography of the sample with authentic arabinose and xylose. They had R_f (arabinose, xylose) : 0·27, 0·27 (BAW) ; 0·55, 0·50 (phenol); 0·45, 0·48 (*t*-BAW) ; 0·30, 0·35 (ethyl acetate : pyridine : water = 10 : 4 : 3) ; 0·25, 0·27 (butanol : ethanol : water = 4 : 1 : 1) and 0·36, 0·44 (benzene : butanol : pyridine : water = 1 : 5 : 3 : 3). Thus, the glycoside was identified as quercetin–3–0–L–arabinoside and a direct comparison, co-chromatography and superimposable I.R. spectra with an authentic compound showed it to be avicularin (quercetin–3–0–α–L–arabinoside).

In order to ascertain whether the quercetin glycoside isolated from *C. ledgeriana* was really quercetin–3–xyloside or the so far misidentified reynoutrin, an attempt made to procure a small quantity of the sample isolated from *C. ledgeriana*[4] for comparison was unsuccessful. The absence of record of the occurrence of quercetin–3–0–xyloside so far in nature, the earlier misidentification of reynoutrin as a xyloside and the present isolation of avicularin from *C. officinalis* and *C. robusta* warrant a re-examination of the true nature of the quercetin pentoside[4] isolated from *C. ledgeriana*[4].

Our grateful thanks are due to Shri S. Kalyanasundaram, Botanist, Government Cinchona Plantations, Dodabetta, Nilgiris, for authentic leaves of *C. officinalis* and *C. robusta,* Drs. T. R. Govindachari and B. S. Joshi, Ciba–Geigy Research Centre, Bombay–63, and Dr. L. Horhammer, University of Munich, Germany, for the spectral data and the Principal, JIPMER, for encouragement.

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THE EFFECT OF TOLUENE, PHENOBARBITAL AND 3-METHYLCHOLANTHRENE ON HEPATIC MICROSOMAL LIPID PEROXIDATION

PHENOBARBITAL AND 3-METHYLCHOLANTHRENE are known to be potent inducers of a range of hepatic microsomal xenobiotic metabolizing enzymes. It has been reported that the pretreatment of rats with either of these compounds produces inhibition of hepatic microsomal lipid peroxidation[1]. Numerous attempts have been made to study the levels of liver lipid peroxides in carbon tetrachloride treated rats[2–4] and it has been reported that the butylated hydroxytoluene[5,6] and benzene[7] significantly reduce the lipid peroxide formation.

Except for a preliminary study[8], involving some aromatic hydrocarbons, there is no recorded information about the effect of organic solvents on hepatic microsomal lipid peroxidation in rat. Accordingly, the present paper reports the effect of toluene on liver microsomal lipid peroxidation. Furthermore, the effect of pretreatment of phenobarbital and 3-methylcholanthrene on the toxicity of toluene was also studied.

Materials and Methods

Hindustan antibiotic strain adult male (200–250 gm) and female (135–160 gm) albino rats were obtained from Hindustan-Antibiotics, Poona. The animals were kept in an air-conditioned room and supplied with rat pellets (obtained from Hindustan-Lever Ltd., Bombay) *ad libitum* prior to the initiation of the experiments. All animals were fed on a synthetic diet for 1 week before initiation of the experiments. The composition of the diet was as reported earlier[9] except that the casein content was increased to 18%. The rats were then allotted to the following groups and pair fed during the experimental period. (1) Control, (2) Toluene treated, (3) Phenobarbital treated, (4) Phenobarbital and toluene treated, (5) 3-Methylcholanthrene treated and (6) 3-Methylcholanthrene and toluene treated. Toluene (0·72 ml/kg body wt.) was administered orally to the rats using corn oil as a carrier in the morning (before feeding) for 2 successive days. Sodium phenobarbital (80 mg/kg body wt.) was injected intraperitoneally, daily, in the morning between 8·0–9·0 a.m. for 3 days,