in cm^{-1}) and PMR on Perkin-Elmer-R-32 spectrophotometer using TMS as internal standard (chemical shifts in δ, ppm). The purity of compounds was checked by TLC on silicagel G-plates and spots were located by I_2 vapours

2-Phenyl-3(4-nitrobenzoyl hydrazono)-methylenylindole (**1**): 2-Phenyl-indole-3-aldehyde (0.01 mol) and 4-nitrobenzoyl-hydrazide (0.01 mol) dissolved separately in ethanol, were mixed and glacial acetic acid (2 drops) was added. The reaction mixture was refluxed for 4 hr, cooled and separated solid recrystallised from alcohol; yield—80%, m.p. = 165°; IR: 3350, 3000, 1670, 1600, 1570, 1510, 1330. PMR (DMSO-d_6): 7.2-7.6 (*m*, 11H, indolyl-4-7*H*, C_6H_5 at position-2 of indole, CON*H* and *H* C = N), 8.2 (*q*, 4H, protons at position 2,3,5 and 7 on phenyl ring at the side chain), 8.7 (*s*, 1H, indolyl N*H*).

Other compounds **2-6** were similarly synthesised (table 1).

2-Phenyl-3-[N(4-nitrobenzoyl), N-benzoyl hydrazono] methylenyl-indole (**7**): Compound **1** (0.003 mol) was taken in aq. NaOH (10%) and benzoylchloride (0.004 mol) was added in fractions, with vigorous shaking. The solid which separated was filtered, washed well with cold water and recrystallised from ethanol yield: 67%; m.p. = 228-30°; IR: 3350, 3050, 1670, 1600, 1510, 1330. PMR: 7.1-7.5 (*m*, 10H, indolyl 4.7*H*, C_6H_5 at position-2 of indole and C*H* = N); 7.95-8.3 (*m*, 9H, CO-C_6H_5 and protons at positions 2,3,5 and 7 on nitro phenyl ring), 8.65 (*s*, 1H, indolyl N*H*).

Compounds 8-12 were similarly synthesised (table 1).

The compounds were given to albino mice of either sex weighing between 20-25 g, at the dose levels of 464, 1000 and 215 mg/kg weight of mice and the mortality rates after 24 hr were noted. From the mortality rate, the approximately lethal dose on 50% of tested animals (ALD_{50}) was calculated by the method of Weil[8].

The compounds were screened out for the antiinflammatory action on mice, following the method of Winder *et al.*[9] measuring the percentage protection of mice against carrageenin induced inflammation at the dose level of 1/5th of the respective compounds.

The compounds were tested for their antibacterial activity[10] against *E. coli*, *X. malvacearum*, *B. megatherium* and *Streptomyces scabies*.

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PEROXODIPHOSPHATE CLEAVAGE BY ALKALINE PHOSPHATASE

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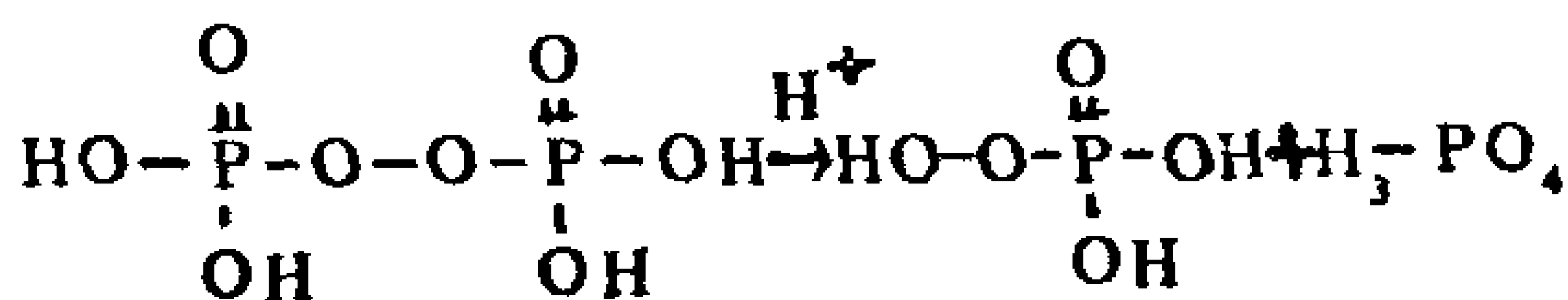
Since the earliest demonstration of phosphatase activity in 1911[1], there have been consistent and sustained efforts to understand the mechanism of this important reaction.[2-4]. Alkaline phosphatase is a nonspecific enzyme, which hydrolyses compounds containing a wide spectrum of phosphate bonds (P—F, P—O—C, P—O—P, P—N and P—S)[3].

We have recently been interested in the electron transfer reactions of peroxomonophosphoric acid[5-8] (H_3PO_5 PMPA), which was obtained by acid catalysed hydrolysis of peroxodiphosphoric acid ($H_4P_2O_8$, PDP).



A preliminary report by FMC[9] that peroxodiphosphate is cleaved by acid phosphatase (wheat flour) and alkaline phosphatase (calf intestine) prompted us to undertake a detailed kinetic and mechanistic investigation of this enzymatic hydrolysis.

Peroxophosphates seemed unique in two ways:

(*a*) The presence of P—O—O—P moiety makes it a potential candidate for the phosphatase activity since the acid catalysed hydrolysis does not occur, or is negligibly slow[10] above pH 2. The O—O and O—P bonds are unusually stable to cleavage; and at 25° C in the range of pH[4-9] in aqueous solutions, there is minimum hydrolysis over a period of one month[9].

(*b*) PMPA is a potential oxidizing agent with germicidal[9] and insecticidal[11] activity. This may well undergo further enzymatic cleavage to H_2O_2 and H_3PO_4.

In this communication we present our results on the remarkably facile enzymatic cleavage of PDP to PMPA by alkaline phosphatase [Phosphomonoesterase, alkaline (EC 3.1.3.1), calf intestine, Sigma]. The enzymatic cleavage has been followed by an iodometric method at pH 4-5, where $H_4P_2P_8$ and H_3PO_5 show differential reactivity towards iodide ion. The liberation of iodine with H_3PO_5 was instantaneous under conditions when the oxidation of I^- by $H_4P_2O_8$ was negligibly slow[12]. This seems to be the most simple and unequivocal demonstration of phosphatase activity.

The pH rate profile of the enzymatic reaction in Tris-HCl buffer indicated a maximum at pH 8 (figure 1), in contrast to the usual *p*-nitrophenyl phosphate cleavage at pH 9.5 under our conditions in carbonate and bicarbonate buffer. The activation energy of enzymatic hydrolysis of PDP (5.7 kcal/mole) is low, when compared to the energy of activation of β-glycerol phosphate (9.94 kcal/mole) hydrolysis[3] by bone alkaline phosphatase at pH 9 and *p*-nitrophenyl phosphate

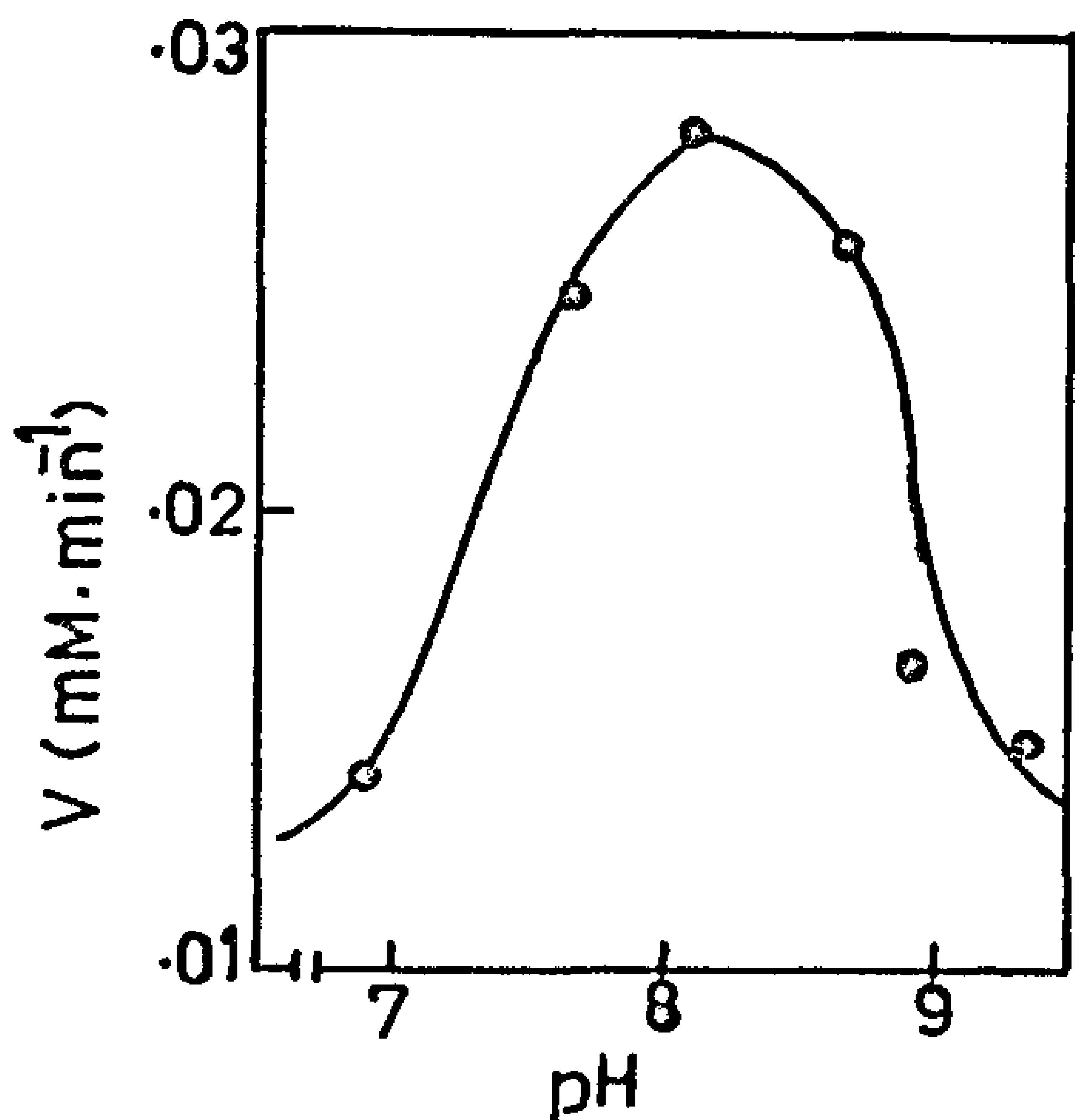


Figure 1. Effect of pH on enzyme catalyzed PDP hydrolysis. ([S] ≅ 1.155 mM)

(10.38 kcal/mole) hydrolysis[3] by placental phosphatase at pH 10.5. It is relevant to point out that the acid catalysed hydrolysis[10] of PDP has an E_a of 18-28 kcal/mole. The K_m value obtained for PDP hydrolysis from a double reciprocal plot (figure 2) at pH 8 is 6.6×10^{-4} M, while the K_m value for *p*-nitrophenylphosphate cleavage is 6.0×10^{-3} M at its pH maxim. The lower K_m for the PDP cleavage is significant and points to a substantial preference of the enzyme for the peroxophosphate.

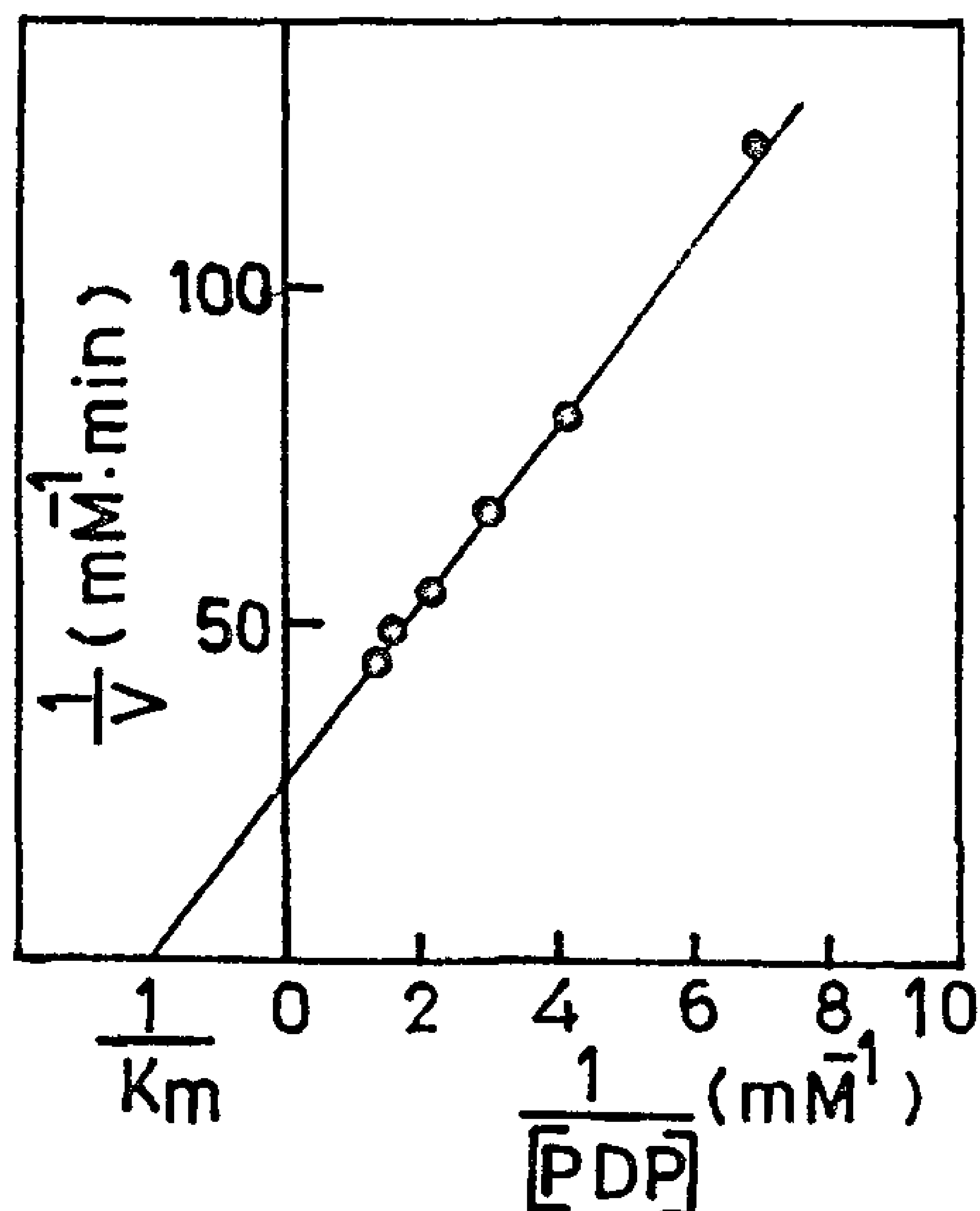


Figure 2. Lineweaver-Burk plot of enzyme catalyzed PDP hydrolysis. (pH 8)

The competitive inhibition of peroxodiphosphate cleavage by phosphate was similar to responses shown with other substrates. The cleavage was completely inhibited by EDTA treatment, and this inhibition was removed by 90% on the addition of an equimolar amount of Zn^{2+} ion. Neither Mg^{2+} nor Ca^{2+} could activate the system; rather they inhibited slightly at higher concentrations.

The presence of a small amount of H_2O_2 in the system has been confirmed by titrating aliquots with $KMnO_4$ and ceric sulphate and is also seen by the stability of the titre in the presence of ammonium molybdate[13]. This could arise by reaction 2.

$$HO-\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}-O-OH \xrightarrow{ENZ} HO-OH + H_3PO_4$$

The biochemical significance of the enzymatic cleavage of PDP is not clear at this moment.

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GENETIC ANALYSIS OF THE TRILOBATE LEAF MUTANT IN MUNGBEAN (*VIGNA RADIATA* VAR. *AUREUS* (L.) WILCZEK)

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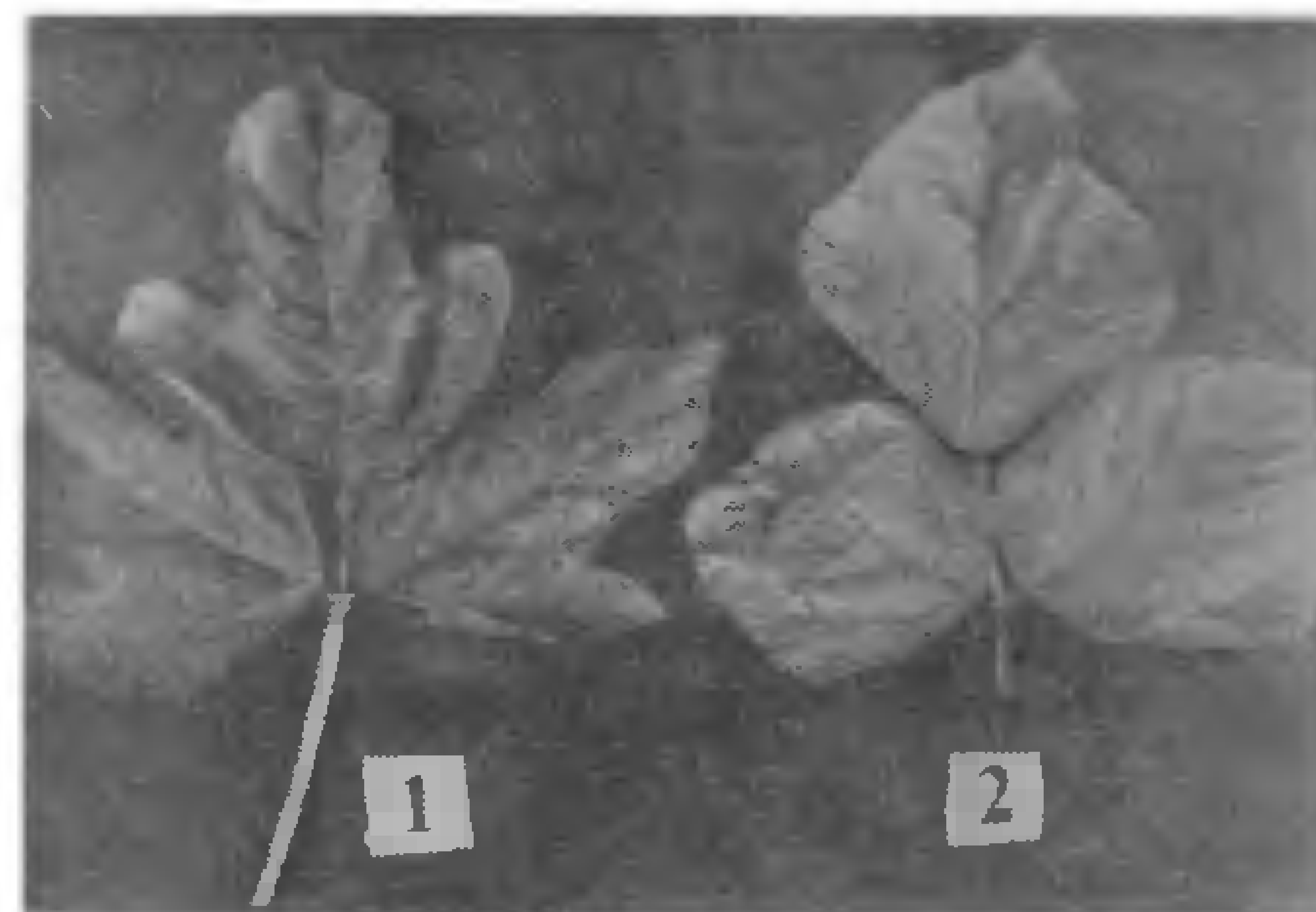
SEEDS of mungbean cultivar K851 were irradiated with different doses of gamma rays (source ^{60}Cc) and from the M_1 generation of the seeds treated with 60 kR dose a leaf mutant was selected. This mutant plant had all trilobate leaves in contrast to the standard plants which had monolobate leaves (figures 1 and 2). A true breeding stock of the mutant was prepared for further studies and the present communication reports the results of the inheritance studies conducted in this mutant line.



Figures 1 & 2. Leaves of the trilobate mutant (1) and the normal (2) plants.

Seeds of the trilobate leaf mutant of mungbean selected in the M_1 generation were grown during the next season and the true breeding ones were selected. These were again tested for segregation in the preceding season in a plant to row system. As there was no further segregation, these plants were marked as true breeding trilobate leaf mutants. The plants of cultivar K851 were used as the standard parent. For making the crosses, the unopened flower buds were emasculated in the evening, immediately sprayed with 50 ppm aqueous solution of kinetin (Sigma, USA) to minimise flower shedding and were pollinated the next morning. Reciprocal crosses were attempted between the mutant and the standard genetic stock and the data recorded on F_1's (both ways), F_2 and the backcross progenies. Cytological studies were also done by squashing young anthers[1].

All the F_1 plants raised from the seeds obtained by reciprocally crossing the mutant types with the standard ones exhibited the mutant phenotype, thus suggesting the dominant expression of the mutant gene over its standard allele. There were, however, no differences in the F_1 progenies of the reciprocal crosses.

The F_1 plants were further crossed to the two parental types and were also allowed to self fertilize. The data presented in table 1 reveal that the F_2 progeny showed a 3:1 segregation ratio typical of the one expected in case of a single dominant gene inheritance. In the backcross progeny of the cross $F_1 \times$ mutant, all the plants were mutant type while in the cross $F_1 \times$ standard, a typical 1:1 segregation ratio was