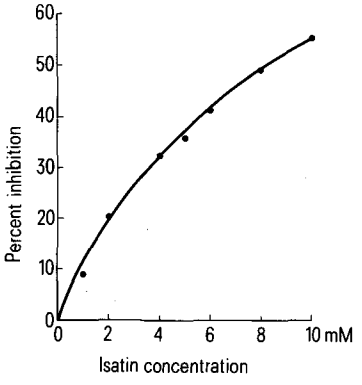

Fig. 1. Inhibition of rat testicular alkaline phosphatase by isatin at different concentrations.

The data on thermodynamic parameters for the enzyme-inhibitor interaction is presented in the table. The value of the apparent association constant, K, was obtained at log (isatin) = -2.0 from figure 3. The molar enthalpy change, ΔH, was found to be -1.038 kcal/mole (figure 3, inset). The entropy change, ΔS, varied from +8.65 to +8.67 e.u./mole with the temperature ranging from 17° to 37 °C. This positive entropy change may be attributed to the unfolding of the protein structure to fit the substrate.

The number of inhibitor molecules combining with 1 molecule of the enzyme was found to be 1.29. Also, the values for the change in entropy and enthalpy were characteristic of non-allosteric inhibition of the enzyme. From these findings, it may be inferred that inhibition of rat testicular alkaline phosphatase by isatin is non-allosteric and non-competitive in nature.

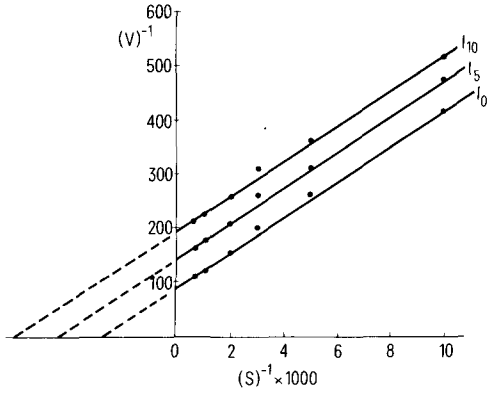

Fig. 2. Line-weaver Burk plot of the effect of substrate concentration. I_0, without isatin; I_5, 5 mM isatin; I_{10}, 10 mM isatin.

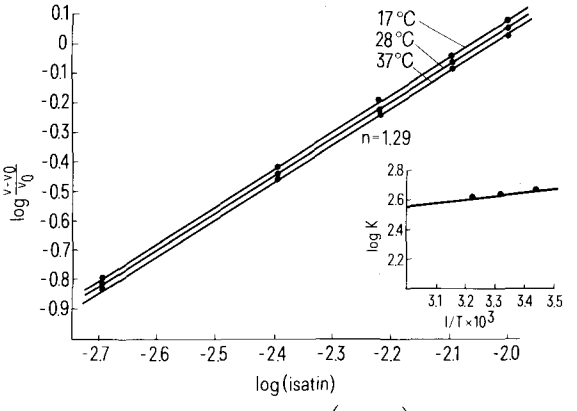

Fig. 3. Plot of log (isatin) versus log $\left(\frac{v-v_0}{v_0}\right)$ at different temperatures.

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Effect of prenatal and neonatal pantothenic acid deficiency on rat intestinal phosphatases

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Summary. Alkaline phosphatase activity was increased in the distal part of the small intestine of pantothenic acid deficient neonatal rats, while acid phosphatase activity was slightly increased and protein concentration was decreased throughout the small intestine. The growth and maturation of the distal part of the small intestine were retarded more severely than in the proximal part.

Pantothenic acid deficiency during gestation results in deranged lipid metabolism of foetuses in guinea-pigs[2]. The acetylating capacity of tissues is markedly reduced in pantothenic acid deficients rats[3]. Deprivation of the vitamin in pigs reduces coenzyme A activity in the mucosa of the intestine[4].

Acute deficiency of the vitamin produces a reduction in flux rate of sodium and mild reduction in the net glucose and potassium transport[4]. Pantothenic acid is also essential to maintain the structural integrity of intestinal mucosa[5,6]. It is also critically required for normal foetal development[7]. The involvement of intestinal alkaline phosphatase in the

absorption of nutrients has been suggested by several authors[8-11]. It is a membrane-bound enzyme localised mainly on the brush border of intestinal mucosa[12,13].

Alkaline phosphatase activity in different segments of the neonatal intestine is age-dependent[14,15]. A study of the enzyme activity in the intestine may give some clue about intestinal maturation in pantothenic acid deficiency. As acid phosphatase is a lysosomal enzyme, its activity in deficiency may reflect lysosomal function. Moreover, it seems that no attempt has been made to study the intestinal phosphatases in pantothenic acid deficiency. So, it is of interest to study the phosphatases of the vitamin deficient intestine.

Materials and methods. Female rats (Charles Forster strain) weighing 190–210 g were fed ad libidum either a purified standard laboratory diet or one deficient in pantothenic acid, right from conception till lactation. The standard diet contained 20% vitamin-free casein, 7 g of groundnut oil, 4 g of salt mixture[16] and 2 g of vitamin mixture[17] and sago prepared from tapioca *(Manihot utilissima)* flour to make up to 100 g. In order to avoid total failure of reproductive performance, an amount of pantothenic acid that results in viable but deficient pups was added to the deficient diet, that was 10% of the estimated requirement (1 mg calcium pantothenate per kg diet). To ensure that results obtained in the vitamin deficient pups are not due to associated undernutrition, neonatally undernourished pups were obtained by increasing the litter size from 8 to 16. Pair fed mother's pups were not used, because, these pups did not significantly differ from normal control pups in growth. Pups from all the 3 groups were killed on 21st day of postnatal life and the intestines were removed.

Preparation of enzyme extract: Each intestine was divided into duodenum, jejunum and ileum (proximal $\frac{1}{3}$ of the total length of the intestine was taken as duodenum, and the rest of the intestine was divided into 3 equal parts and the 1st part was taken as jejunum and the rest as ileum). A 10% extract (w/v) of each segment was prepared in ice-cold glass distilled water by homogenizing the tissue in a Potter Elvenhjem homogenizer for 2 min at 0–2 °C, at 5000 rpm and filtered through cotton wool. The filtrate was used as crude enzyme extract.

Assay system for phosphatases: The assay system for alkaline phosphatase consisted of 10 mM p-nitrophenyl phosphate (Sigma), 10 mM magnesium chloride, 200 mM carbonate bicarbonate buffer (pH 9) and 0.1 ml of enzyme extract.

The assay system for acid phosphatase consisted of 10 mM p-nitrophenyl phosphate, 50 mM sodium acetate-acetic acid buffer (pH 3.5) and 0.2 ml of enzyme extract. The final volume of the assay system was made up to 5 ml with glass-distilled water. The reaction was started by the addition of the enzyme extract and the incubation was carried out at 37 °C (alkaline phosphatase) or 60 °C (acid phosphatase) for 15 min. The reaction was terminated by the addition of 5 ml 10% (w/v) TCA. In the blank, TCA was added prior to incubation. The liberated phosphorus was estimated by the method of Fiske and Subbarow[18]. The enzyme unit is defined as the amount of enzyme required to liberate 1 µmole of phosphorus per min under the assay conditions. The protein content of the tissue was determined according to the method of Lowry et al.[19] with bovine serum albumin as a standard.

Results and discussion. Body and intestinal weight of pups were significantly reduced both in pantothenic acid deficiency and undernutrition. But the deficit in b.w. was more in the case of undernourished pups (table 1).

A proximal-to-distal gradient was observed in intestinal weight reduction of the vitamin-deficient animals. The distal portion of the intestine was more reduced and the proximal part was less affected (table 1). In the case of neonatal undernutrition, percentage deficit in weight was the same in duodenum and jejunum, while in ileum it was more than in the other segments (table 1). Although b.w. retardation of pups was less in pantothenic acid deficiency than in undernutrition, the deficit in the distal part of the intestinal weight was more in the vitamin-deficient pups. This shows a greater sensitivity of the distal part of the intestine to pantothenic acid deficiency than that of neonatal undernutrition.

The protein concentration showed a mild reduction in duodenum and jejunum of both the groups in comparison with controls. In ileum, protein concentration was not significantly altered in undernourished pups whereas it was slightly reduced in the vitamin-deficient pups suggesting the more sensitivity of ileum to pantothenic acid deficiency.

Table 1. Effect of prenatal and neonatal pantothenic acid deficiency on body and intestinal growth of neonatal rats

	Control (a)	Pantothenic acid deficient (b)	Undernourished*** (c)	$\frac{b}{a} \times 100$	$\frac{c}{a} \times 100$
Body weight (g)	42±0.3	28.5±2.8* ($p<0.01$)	20.5±0.5*	67	49
Duodenal weight (g)	0.42±0.015	0.24±0.025*	0.21±0.007*	58	50
Jejunal weight (g)	0.52±0.013	0.23±0.023* ($p<0.01$)	0.27±0.01*	44	50
Ileal weight (g)	1.13±0.03	0.31±0.021* ($p<0.001$)	0.47±0.01*	27	41
mg Protein/g wet tissue					
Duodenum	127±1	119±1.1*	118.7±2.1*	94	93
Jejunum	131±1	118±0.7* ($p<0.05$)	123.5±2**	90	94
Ileum	121±1.5	112±0.8*	115.5±2.3	92	96

Values are based on observations made on 6-8 rats in each group (mean±SE). Values marked with asterisk significantly different from control values, *$p<0.001$; **$p<0.01$; ***Neonatal undernutrition was induced by increasing the litter size from 8 to 16. p values obtained when compared with undernourished pups are given in parenthesis.

Table 2. Effect of prenatal and neonatal pantothenic acid deficiency on intestinal alkaline phosphatase

Segment	Control (a)	Pantothenic acid deficient (b)	Undernourished*** (a)	$\frac{b}{a} \times 100$	$\frac{c}{a} \times 100$
Alkaline phosphatase activity					
Units/g tissue					
Duodenum	52 ± 3	53 ± 1.9 ($p < 0.05$)	48 ± 1*	102	92
Jejunum	45 ± 1.1	53 ± 1.58* ($p < 0.01$)	48 ± 0.6**	118	106
Ileum	33 ± 0.26	52 ± 1.9* ($p < 0.001$)	40.8 ± 1.9*	158	123
Units/mg proteins					
Duodenum	0.41 ± 0.005	0.46 ± 0.02** ($p < 0.05$)	0.41 ± 0.01	112	100
Jejunum	0.34 ± 0.006	0.46 ± 0.015* ($p < 0.01$)	0.40 ± 0.011*	135	118
Ileum	0.27 ± 0.002	0.46 ± 0.026* ($p < 0.001$)	0.35 ± 0.014*	170	130

Values marked with asterisk significantly different from control values. *$p < 0.001$; **$p < 0.05$ (mean ± SE). Values are based on 6–8 determinations. ***Undernutrition was imposed by increasing the litter size from 8 to 16. p values obtained when compared with undernourished pups are given in parenthesis.

However, the difference in protein concentration of ileum, between the vitamin deficient and undernourished animals, fell short of statistical significance (table 1).

Alkaline phosphatase activity was increased in jejunum and ileum in a manner unlike undernourished pups (table 2). During the postnatal development of the enzyme there is a sharp fall of the enzyme activity in the distal part of the intestine at the end of 3rd week of postnatal life, while the duodenal enzyme activity increases progressively during neonatal development[14,15]. This anterior-to-posterior gradient which is typical for alkaline phosphatase activity of weanling and adult rats was abolished in the vitamin-deficient animals (table 2). In contrast to pantothenic acid deficiency, undernutrition showed a mild reduction of duodenal alkaline phosphatase (activity/g wet tissue).

The activity observed in different segments of the intestine of control pups agrees well with that of B-glycerophosphatase activity studied by Moog and Yeh[15]. The rate of hydrolysis of B-glycerophosphate and p-nitrophenyl phosphate by duodenal alkaline phosphatase are found to be more or less the same[20].

Table 3. Effect of prenatal and neonatal pantothenic acid deficiency on intestinal acid phosphatase activity

Segment	Control (a)	Pantothenic acid deficient (b)	$\frac{b}{a} \times 100$
Units/g tissue			
Duodenum	4.66 ± 0.15	4.95 ± 0.087	106
Jejunum	5.46 ± 0.14	5.48 ± 0.06	100
Ileum	4.37 ± 0.033	4.48 ± 0.102	103
Units/mg protein			
Duodenum	0.038 ± 0.001	0.04 ± 0.0008**	108
Jejunum	0.041 ± 0.0007	0.047 ± 0.0005*	115
Ileum	0.037 ± 0.0006	0.040 ± 0.008**	108

Values are based on 6–8 determinations. Values marked with asterisk are significantly different from control values. *$p < 0.001$; **$p < 0.05$.

The present studies on alkaline phosphatase activity strongly suggest that some of the age-dependent development and associated biochemical changes are retarded or delayed, in particular, in the proximal part of the intestine of the vitamin-deficient animals. The biochemical changes responsible for the fall of the phosphatase activity in ileum at weaning would have been delayed. This retardation is less severe in the undernourished rats. Acid phosphatase activity (per mg protein) showed a tendency to decline, which suggests an increased lysosomal catabolism of the tissue as the enzyme is a lysosomal one.

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