This research has pointed the relations between chlorogenic acid content and age of the plant or organ, changes in composition of the orthodiphenol fraction in function of the physiological stage, changes in composition of the orthodiphenolic fraction between the various organs and metabolism of the various components.

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Differential developmental pattern of acid and alkaline phytase and phosphatase activities in rat intestine

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Summary. Rat intestine was found to show a distinct acid phytase activity (pH optimum 4.7) in addition to that of an alkaline phytase (pH optimum 8.0). The phytase and phosphatase activities were found to differ in their developmental pattern and responded differentially to some inhibitors. Thus the two activities seem to be due to two independent enzymes and are not the activity of a nonspecific phosphatase as has been suspected formerly.

The enzyme phytase (EC 3.1.3.8) with an alkaline pH optimum has been reported[1-4] in the intestine of various species including man. Our preliminary studies[5] indicated the presence of an acid phytase, in addition to that of the alkaline phytase in rat intestine. Studies were therefore undertaken to confirm the presence of 2 distinct phytase activities in rat intestine. The attempts were also made to differentiate the phytases from corresponding phosphatases on the basis of their developmental patterns and the response to various inhibitors.

Materials and methods. Method for the preparation of tissue extract and assay of the enzyme activities was as reported by Bitar and Reinhold[4] and modified as described earlier[6]. Protein was estimated by Lowry's method[7] and phosphorus by that of Fiske and Subbarao[8]. For the developmental studies intestines of normal healthy rats (Charles Foster) of different ages (7–10 in each case) were used.

Results and discussion. The initial studies were carried out to determine the optimum conditions for the assay of intestinal phytase and phosphatase activities, using intestinal mucosal extract of adult rat as the enzyme source. The pH curve (figure 1) for phytase showed 2 distinct peaks, a small one in the acid range at pH 4.7 and a large one in the alkaline range at pH 8.0, indicating the presence of 2 phytases. On the other hand, phosphatase showed pH optima at 3.8 and 9.0. The optimum conditions worked out for the acid and alkaline phytases and phosphatases are given in table 1. To confirm the presence of 2 distinct phytases in the intestine, attempts were made to separate acid phytase activity from that of alkaline phytase in intestinal mucosal extract. For this purpose, intestinal mucosal extract (10% w/v) was subjected to acid treatment by bringing its pH to 4.0 with 0.1 M acetic acid and after 1 h the extract was centrifuged at 10,000 × g for 40 min. The pH of so obtained supernatant and residue was readjusted to 7.6 with 0.1 M Na_2CO_3. The acid phytase activity

Table 1. Optimum assay conditions for phytase and phosphatase activities of rat intestine

	Acid phytase	Alkaline phytase	Acid phosphatase	Alkaline phosphatase
Optimum pH	4.8	8.0	3.7	9.0
Substrate concentration (mM)	0.8	0.8	0.8	1.6
Enzyme concentration (mg protein)				
Intestinal mucosa	1.50	1.50	1.50	0.75
Intestine whole	2.2	2.2	2.2	1.1
Optimum temperature (°C)	60	60	60	37
Linearity till (min)	15	15	15	15

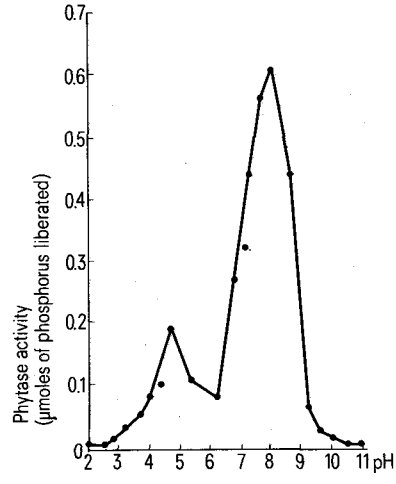

Fig. 1. pH curve of phytase activity of rat intestinal mucosa. Buffers used: glycine-HCl, for 2.0–3.5 pH, acetate for 3.5–6.0 pH tris succinate for 6.0–9.0 pH, and carbonate bicarbonate for 9.0–11.0 pH.

Table 2. Partial purification of acid phytase from rat intestinal mucosa

Fraction	Total activity[b] Acid phytase (units)	Alkaline phytase (units)	Specific activity[b] Acid phytase	Alkaline phytase	Ratio of specific activity of acid phytase to alkaline phytase
Crude	7.31	15.73	0.013	0.027	0.48
pH 4 supernatant[a]	2.17	0.59	0.053	0.014	3.8
pH 4 residue[a]	4.28	14.08	0.001	0.032	0.03
60–75% ammonium[a] sulfate precipitate	0.76	0.03	0.146	0.007	20.8

[a] pH of crude homogenate was adjusted to 4 and after 1 h, supernatant and residue were collected by centrifugation and pH was readjusted to 7.6. The protein of supernatant precipitated between 60–75% saturation of ammonium sulfate were collected by centrifugation and suspended in water and dialyzed. [b] Amount of enzyme required to liberate 1 µmole of phosphorus per min from the substrate under assay condition is equivalent to 1 unit of the enzyme. Unit of enzyme per mg protein is expressed as specific activity.

present in the supernatant was associated with a minimum of alkaline phytase activity. The protein precipitated from the supernatant between 60 and 75% saturation of ammonium sulfate was collected and dialyzed against distilled water. This fraction was found to contain acid phytase activity 11-fold of the crude, with a little alkaline phytase activity. The ratio of acid phytase to alkaline phytase in this fraction rose to 20.8 from 0.48 in the crude extract (table 2). The partial separation of acid phytase from that of alkaline phytase achieved by this method suggests the presence of 2 distinct phytases in rat intestine. The presence of acid phytase indicated by this investigation is of special interest, since the earlier studies[1–4] have reported only alkaline phytase in the intestine.

Normal developmental pattern of phytases and phosphatases was found by determining their activity in the intestine of rats at different ages, and this is given in figure 2. Acid and alkaline phytase activities increased in late fetal period followed by a decrease at birth. Acid phytase activity remained stationary in neonatal period followed by a slow decline afterwards. Alkaline phytase, on the other hand, increased after birth to 63 days of age and then decreased. Alkaline phosphatase followed the same pattern as that of alkaline phytase up to 2nd week of age but decreased in the 3rd week and then remained unaltered. Acid phosphatase showed a high activity in the 1st week and decreased considerably in the 2nd week and again showed a slight rise in the 3rd week. In postweaning period, there was not much change in the activity of this enzyme whereas acid phytase showed a decrease.

The differential developmental patterns of acid and alkaline phytases support their distinct identity. The differences

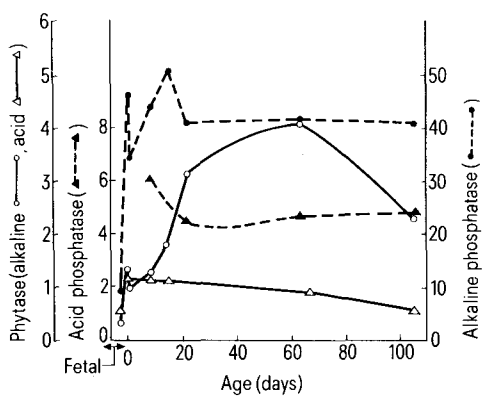

Fig. 2. Developmental pattern of phytase and phosphatase activities (unit/g) in rat (Charles-Foster) intestine (7–10 observations in each case). Fetal and at birth values for alkaline phosphatase have been taken from Subramoniam[13] (the parallel studies carried out in this laboratory on the same strain of rats under similar experimental conditions).

in certain characteristics, as well as in the developmental pattern of phytases from corresponding phosphatases, suggest that the 2 activities (phytases and phosphatases) are not due to a nonspecific phosphatase, as was suggested earlier by Pileggi[9] and Maddaih et al[10]. Their differential developmental pattern is also suggestive of differences in their role during development. Acid and alkaline phosphatase activities during development have been shown to be associated with changes in the cellular differentiation and histogenesis[11,12]. In this connection, our earlier studies[6] have shown a relationship between intestinal maturation and its phytase activities. Recent studies in this laboratory (Ramakrishnan, Bhandari, Renuka Chandrasekhar and Rawal, unpublished) have indicated that phytases may have a role in the phosphoinositide turnover of the cells, as has been discussed by us earlier[6]. Further studies are in progress to purify these enzyme and study their role in intestinal metabolism.

Further, an attempt was made to differentiate phytase and phosphatase activities on the basis of the effects of some inhibitor substances (1 mM) on their activities. Mesoinositol was found to inhibit only alkaline phytase (19%). Degree of inhibition due to EDTA was more pronounced in the case of alkaline phosphatase (99%) than of alkaline phytase (70%). On the other hand, PCMB (0.2 mM) inhibited acid phytase (100%) more than acid phosphatase (78%). p-Nitrophenol inhibited only phytases, the effect being greater on acid phytase (61% against 20% in case of alkaline phytase). Alkaline phosphatase was not inhibited by either oxalate or citrate, whereas a mild to moderate (19–57%) effect was found in the case of other enzymes. Only alkaline phosphatase was inhibited by sodium cyanide (33%). The differential inhibition of phytases and phosphatases by different inhibitors also suggests that these activities are not due to a nonspecific phosphatase and are associated with independent enzymes.

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