The observations reported here indicate that the effectiveness of an antimetabolite in inhibiting a normal growth process may be altered by changes in the intensity of the growth stimulus. A similar principle may conceivably apply to neoplastic growth. If so, there are implications with respect to possible benefits of combination therapy in certain types of tumors.

Summary. The growth-stimulating effect of testosterone on the seminal vesicle of the castrated rat is inhibited by 5-fluorouracil. The extent of this inhibition diminishes with increase of either hormone dosage or duration of treatment, and may be overcome completely by appropriate amounts of hormone at all tolerated levels of fluorouracil administration.

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Nature of Intestinal Phytase Activity.* (29114)

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Phytases, enzymes hydrolyzing phytic acid, are widely distributed in animal tissues. Phytase activity was first observed in the intestinal mucosa of rats(1) and later in the chicken, pig and cow(2). Phytase activity has been demonstrated to be widely distributed in the rat, the highest activity being found in the proximal part of the intestinal mucosa(3).

An increase has been reported in the intestinal phytase activity of rats and chicks kept on cereal rachitogenic rations when supplemented with vitamin D(4). Pilleggi *et al* (5) have observed identical responses in phytase and alkaline phosphatase activities in intestinal extracts of rats fed Vit. D. However, no consistent correlation, in a quantitative sense, between degree of phytate hydrolysis by the intestinal homogenates *in vitro* and calcification could be found(6). This suggested that a non-specific phosphatase may act on the phosphate groups of phytates. In the present work, the effect of feeding dicalcium phosphate and sodium and calcium phytates on intestinal phytase activity in rats, chicks and hens and the effect of fluoride on intestinal phytase and alkaline phosphatase activities has been studied. The results of fluoride inhibition and mixed substrates on phytase and alkaline phosphatase activities were used to examine the enzyme(s) involved(7).

Methods and materials. Dietary phosphorus levels in the range of 0.2-0.6% phosphorus, supplied as dicalcium phosphate, were fed to day-old chicks, mature hens and weanling rats for experimental periods of 4, 2 and 6 weeks, respectively. In addition, either sodium or calcium phytate was fed at levels

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TABLE I. Effect of Dicalcium Phosphate, Sodium Phytate and Calcium Phytate on Intestinal Phytase Activity of Chicks, Hens, and Rats.*

Source	Micromoles of phosphate liberated/g tissue/2 hr × 10^{-1}		
	Chicks‡	Hens‡	Rats‡
$CaHPO_4 \cdot 2H_2O$ (all levels)	26.7 ± 3.6†	13.4 ± 2.5	24.8 ± 7.2
Calcium phytate " "	32.0 ± 5.0	13.6 ± 2.2	—
Sodium " " "	—	12.8 ± 3.0	18.7 ± 8.0

* .04 M sodium phytate as substrate.
† ± standard deviation of the mean.
‡ Intestinal homogenate source. Each value represents 10 animals.

to supply 0.4-0.9% phosphorus as shown in Table I. The chicks were housed in electrically heated batteries with raised wire floors and fed the experimental diets *ad libitum*. The laying hens were housed in individual cages and the rats were maintained in individual hanging cages throughout the experimental period. The basal diet used was the blood fibrin diet of Gillis and associates (8).

At the end of each experimental period, a representative sample of the animals in each group was sacrificed by mild anesthesia and the small intestine removed. Intestinal homogenates (10%) were prepared in a Potter and Elvehjem glass homogenizer from a washed and weighed portion of the duodenum.

All enzyme assays were carried out by incubating 0.5 ml of the homogenate in a total volume of 5 ml reaction mixture at pH 7.8 (2,10) and 37.5 ± 0.5°C for a period of 2 hours. The reaction was then stopped by addition of 5 ml of 10% trichloroacetic acid, filtered and aliquots taken for inorganic phosphate determinations by the method of Fiske and Subbarow(9). Enzyme activities were expressed in terms of inorganic phosphorus liberated per gram of wet tissue in 2 hours.

Substrates used were sodium phytate§ for phytase assays and beta-glycerophosphate‖ for alkaline phosphatase assays. Substrate solutions, both for phytase and alkaline phosphatase assays were 0.04 M with respect to organic phosphorus. Sodium phytate contained 2.6% inorganic phosphorus. To duplicate exactly the conditions of phytase assay, inorganic phosphate was added to the beta-glycerophosphate substrate in an amount equal to that introduced by the inorganic phosphorus present in the sodium phytate substrate solution. For inhibition assays suitable volume of 0.5 M sodium fluoride was added to the reaction solution. In the study of the mixed substrate effect, a mixture of 1.0 ml of each of the 2 substrates was used.

Results and discussion. No significant differences in intestinal phytase activities of chicks, mature hens or rats were obtained with the feeding of any of the dietary phosphorus levels employed or with the dicalcium phosphate ($CaHPO_4 \cdot 2H_2O$), sodium phytate or calcium phytate supplements. These data confirm the earlier report of Roberts and Yudkin(6) to the extent that intestinal phytase activity was not increased as a result of adding phytate to the diet of rats. These workers, as well as others, noted an increase in the activity of this enzyme when the dietary Vit. D level was raised. Our studies employed no Vit. D treatments and the level of this vitamin in the respective diets was held constant.

The lack of a relationship between the *in vitro* intestinal phytase activity and the well established differences in utilization of sodium and calcium phytates compared to dicalcium phosphate should also be noted in comparing the intestinal phytase activities of the experimental animals employed (Table I). Both chicks and rats showed the same degree of *in vitro* intestinal phytase activity while laying hens in this study exhibited only about one-half of the activity obtained with chicks and rats.

Gillis *et al*(11) have reported that phytin phosphorus utilization by laying hens was only one-half that of dicalcium phosphate,

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TABLE II. Effect of Mixed Substrate and Fluoride Concentration on Intestinal Enzyme Activity *in vitro*.

Substrate	Fluoride concentration (M)	Micromoles phosphate/g tissue/hr $\times 10^{-1}$
Chicks		
Sodium phytate (0.04 M)	.00	29.9 ± 6.7*
	.01	19.5 ± 4.4
	.05	13.2 ± 4.3
Beta-glycerophosphate (0.04 M)	.00	70.7 ± 5.9
Sodium phytate (0.04 M) + beta-glycerophosphate (0.04 M)	.00	69.0 ± 10.6
Hens		
Sodium phytate (0.04 M)	.00	13.3 ± 3.5
	.01	11.9 ± 3.0
	.05	8.6 ± 3.0
	.10	4.0 ± 3.6
Beta-glycerophosphate (0.04 M)	.00	51.9 ± 8.7
	.01	49.2 ± 12.4
	.05	29.7 ± 10.9
	.10	16.0 ± 5.5

* ± standard deviation of mean. Each mean represents 10 samples.

and in an earlier study(12) these workers indicated that the chick was able to utilize phytin phosphorus to a lesser extent than the mature hen.

A study of the existence of a specific phytate splitting enzyme in intestinal homogenates was also undertaken. Using a mixed substrate of equi-molar concentrations of sodium phytate and beta-glycerophosphate compared to the activity obtained with the 2 substrates singly, it was concluded that the phytase activity was due to a non-specific phosphatase (Table II). The activity obtained with the mixed substrate was no more than that with the beta-glycerophosphate alone; if 2 enzyme systems were involved, a higher rate of phosphate hydrolysis should have occurred in the presence of the combined substrates(13).

A level of 0.01 M fluoride produced a 53% inhibition of phytase activity in chick intestinal homogenates. This level of fluoride (as NaF) produced only 10% reduction in activity with the sodium phytate substrate in intestinal homogenates from mature hens. A higher level of fluoride was required to produce the same degree of inhibition in laying hens as compared with chick homogenates (Table II). Additional evidence that the phytase activity was due to a non-specific phosphatase was obtained through the use of fluoride inhibition. The ratio of activities obtained with the 2 substrates in absence of fluoride was 3.9 (beta-glycerophosphate to sodium phytate). In the presence of the fluoride, ratios were 4.1, 3.5 and 4.0, respectively, for the 0.01, 0.05 and 0.01 M fluoride levels. If the phytase and alkaline phosphatase activities had been due to two different enzymes, this constancy of the ratios of the activities obtained with and without the fluoride inhibitor would not have occurred, since it is unlikely that the two enzymes would be inactivated to the same degree under these conditions.

Summary. Experiments were carried out with chicks, mature hens and weanling rats fed dicalcium phosphate, calcium phytate and sodium phytate as dietary phosphorus sources. Intestinal phytase activity measurements failed to show a significant difference in the activity of this enzyme due to the dietary treatments. The mature hen exhibited lower intestinal phytase activity than did the chick or the rat. The use of mixed substrates, employing sodium phytate and beta-glycerophosphate at equi-molar concentrations, failed to produce an increase in activity as compared to these substrates singly. These data indicated that the phytase activity may have been due to a non-specific phosphatase in the intestinal homogenates. The ratios of alkaline phosphatase to phytase activities in presence or absence of fluoride were the same. These data also point to the presence of only one enzyme system which hydrolyzed both sodium phytate and beta-glycerophosphate.

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Feeding Frequency: A Factor in Dietary Protein Utilization.* (29115)

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With a constant food intake, the rate of ingestion of the diet (periodicity of food consumption or load of nutrients to be handled per unit of time) alters the intermediary metabolism of fat and carbohydrate(1,2,3). With fewer meals, an increased percentage of dietary calories is retained as body fat. The manner in which the body handles dietary protein is likewise subject to the influence of feeding frequency; a decrease in number of daily feedings results in the organism depositing less protein and water and excreting more urinary nitrogen(4). Presumably these changes occur as a result of enzymatic adaptations. The results of the present experiments have yielded data consistent with the concept that there is a limit to the quantity of ingested amino acids that can be handled per unit of time by an organism for anabolic purposes.

Methods. In the 2 experiments to be described, male Holtzman rats, received when 100-120 g in weight, were given a moderate carbohydrate-20% protein-diet(4), either *ad libitum* in solid form or in a distilled water homogenate by stomach tube for one week. Force feeding was carried out before 8:45 A.M. and after 4:15 P.M.; half the day's allotment of nutrients was given on each occasion. A stomach tube, but no food, was passed into the animals with free access to the diet at the same times. This protocol served to accustom to the diet and "tubing" the rats with free access to food and to adapt the force-fed ones to the procedure. Distilled water was freely available at all times to all animals.

In the first experiment, the influence of feeding frequency on urinary excretion of N^{15} after the ingestion of N^{15} labelled protein was evaluated. After the week of adaptation, 3 animals eating *ad libitum* and 3 force-fed ones were individually housed in metabolism cages for 5 days. On the sixth day, each animal with free access to food was given the usual diet into which was incorporated a trace amount of N^{15} enriched yeast protein. On the next 2 days, the basic diet without tracer was fed; the amount of diet consumed by each animal was weighed for each of the 3 days. The twice per day "tubing" procedure was continued during this period. The tube-fed rats were individually pair-force-fed against a control animal a homogenized liquid diet that contained 0.7 g of diet per ml. Urine was collected from all animals daily, from 9 A.M. to 9 A.M. from the force-fed ones and from 2 P.M. until 2 P.M. from those eating *ad libitum.* These periods were chosen because of the different eating times (night *vs* day) of the 2 groups. The urines were analyzed for urea N on the Autoanalyzer(5) and for urea N^{15} by mass spectrometry to an accuracy of 0.1%. The

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