Effects of Dietary Fats, Calcium, and Phosphorus on Rat Serum Alkaline Phosphatases* (33518)

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It has long been recognized that a fat enriched diet will result in an increased alkaline phosphatase activity of serum, lymph, and intestinal mucosa of rat (1, 2) and man (3). Effect of the nature of fat, however, was not evaluated.

It was also shown that a low calcium diet leads to an increase in serum alkaline phosphatase activity of sheep (4) and poultry (5). No attempts were made to differentiate the source of increased enzymes.

Fishman *et al.* (6, 7) found that L-phenylalanine specifically inhibits intestinal alkaline phosphatase of human and rat. Using 5 m*M* L-phenylalanine, these investigators observed 78% inhibition of intestinal alkaline phosphatase of man and to a lesser extent that of rat. Based on these studies they concluded that the intestinal isoenzyme contributed 20–45% of the total serum alkaline phosphatase in man. In the rat most of the serum alkaline phosphatase originates from the striated border of the epithelial cells of intestine (8), and thus may be involved in absorptive processes.

The present study was undertaken to study the effect of the levels of dietary fat, calcium, and phosphorus and of the nature of fat on the activity of total and L-phenylalanine-sensitive component of serum alkaline phosphatase of the rat.

Materials and Methods. In all three experiments, weanling male rats of the Holtzman strain weighing 47–60 g were ear-notched and alloted to individual cages. Distilled water was provided *ad libitum* but only 7 g of diet per rat per day in the first two experiments and 8 g in the third experiment were allowed so that they consumed all the diet. The animals were maintained on the experimental diets for 1 week to adapt to the regimen and environment. They were then transferred to metabolism cages and tail cups were affixed to prevent coprophagy. Tail cups were emptied daily, the 7-day fecal collection for each rat was pooled, weighed, and kept frozen until analysis.

In the first experiment 210 rats were divided into 42 groups of 5 each. The composition of the basal diet is given in Table I. The fat content of the diets was 0, 5, or 25% triolein, tripalmitin, or tristearin. With each level and type of fat three levels of calcium (0.08, 0.50, 2.06%) and three levels of phosphorus (0.15, 0.58, 1.67%), giving six calcium-to-phosphorus ratios (0.05, 0.57, 0.87, 1.23, 3.52, 13.75) were used. Calcium was added as calcium carbonate and phosphorus as potassium monobasic phosphate. All the additions were made at the expense of cerelose.

In the second experiment 20 rats were divided into four groups and fed diets containing 25% tristearin alone or supplemented with 5% triolein, 5% monoolein, or 2.5% monoolein plus 2.5% oleic acid.

In the third experiment 30 rats were divided into six groups. Three of the six diets contained tripalmitin at 10, 20, or 30% levels. In the other 3 diets 5% triolein replaced 5% tripalmitin in each case.

TABLE I. Composition of the Basal Diet.

Ingredients	% of the diet
Casein	20.0
Vitamin mixture[a]	2.2
Ca-free salt mixture[b]	4.0
Celluflour	5.0
Chromic oxide	0.2
Cerelose	68.6

[a] Nutritional Biochemicals Co., Cleveland, Ohio.

[b] Mann Research Laboratories, Inc., New York, N.Y.

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At the end of each experiment, the rats were lightly etherized and blood taken by decapitation. The pooled blood of rats in each group was centrifuged, serum was removed and kept frozen until analysis.

Total serum alkaline phosphatase was determined by the Sigma method using *p*-nitrophenylphosphate as substrate. L-Phenylalanine-sensitive component of serum alkaline phosphatase (intestinal) was determined on an aliquot of the serum using the same procedure with the incorporation of 5 m*M* L-phenylalanine in the final incubation mixture for the enzyme determination. Total dietary and fecal fat were determined by means of a toluene extraction procedure using the

TABLE II. Relationship of Fat Absorption to Total and Intestinal Serum Alkaline Phosphatase.[a]

	Fat (g/7 days)			Serum alkaline phosphatase		
Treatment (%)	Intake	Absorbed	Fecal	Total (Sigma units)	Intestinal (Sigma units)	% Intestinal
Triolein (5)	2.45	2.25 ± 0.04	0.20	7.76 ± 0.79	4.11 ± 0.73	52.96
(25)	12.25	11.54 ± 0.24	0.71	29.63 ± 4.22	17.60 ± 2.11	59.40
Tripalmitin (5)	2.45	0.76 ± 0.05	1.69	4.07 ± 0.61	1.63 ± 0.31	40.05
(25)	12.25	3.22 ± 0.06	9.03	8.00 ± 0.71	3.83 ± 0.71	47.88
Tristearin (5)	2.45	0.55 ± 0.06	1.90	4.84 ± 0.54	2.20 ± 0.36	45.45
(25)	12.25	1.93 ± 0.45	10.32	5.29 ± 0.64	2.45 ± 0.33	46.31

[a] Average of 30 rats ± SE of mean.

TABLE III. Effect of Supplementation of Tristearin with Oleic Derivatives on the Activity of Total Serum and "Intestinal" Alkaline Phosphatases.

	Serum alkaline phosphatase			Fat (g/wk/rat)		
Treatment[a] (%)	Total (Sigma units)	Intestinal (Sigma units)	% Intestinal	Intake	Fecal	Absorbed
25 TS	4.80	1.30	27.08	12.25	11.30	0.95
25 TS + 5 TO	8.40	3.80	45.24	14.70	11.15	3.55
25 TS + 5 MO	8.20	3.00	36.59	14.70	10.98	3.72
25 TS + 2.5 MO + 2.5 OA	9.80	4.50	45.92	14.70	10.69	4.01

[a] Abbrev.: TS = tristearin; TO = triolein; MO = monoolein; and OA = oleic acid.

TABLE IV. Effect of Level of Tripalmitin and Its Partial Substitution with Triolein on the Activity of Total Serum and "Intestinal" Alkaline Phosphatases.

	Serum alkaline phosphatase			Fat (g/wk/rat)		
Treatment[a] (%)	Total (Sigma units)	Intestinal (Sigma units)	% Intestinal	Intake	Fecal	Absorbed
10 TP	7.20	4.20	58.33	5.6	3.60	2.00
5 TP + 5 TO	9.00	5.20	57.78	5.6	0.90	4.70
20 TP	9.00	6.65	73.89	11.2	8.70	3.70
15 TP + 5 TO	18.36	11.45	62.36	11.2	3.45	7.75
30 TP	14.40	9.00	62.50	16.8	11.71	5.09
25 TP + 5 TO	19.60	12.20	62.24	16.8	7.98	8.82

[a] Abbrev.: TP = tripalmitin; and TO = triolein.

Wistreich apparatus, fecal and serum calcium by atomic absorption spectrophotometry, and phosphorus by a modified Fiske and Subbarow method (9).

Results and Discussion. The relationship between amount of fat absorbed and activity of total serum and L-phenylalanine sensitive "intestinal" alkaline phosphatases in all three experiments were in good agreement and are summarized in Tables II–IV. The activity was highest in groups receiving triolein, which was almost completely absorbed (97–99%), lowest in rats fed tristearin, which was poorly absorbed (17–29%) and intermediate in groups fed tripalmitin (19–47%). Thus, the rise in activity of this enzyme is not merely a function of fat content of the diet but of the absorbability of fat. The correlation between amount of fat absorbed and activity of total and "intestinal" alkaline phosphatase was highly significant ($r = 0.92$ and 0.89, respectively for the first experiment; 0.97 and 0.92 for the second experiment; 0.95 and 0.94 for the third experiment; p in all cases was <0.001).

It is well recognized that products of fat digestion, monoglycerides, and fatty acids, combine with bile salts to form micelles, the form which is believed to be brought to the brush border of intestinal mucosa for absorption. Therefore 5% triolein, 5% monoolein or 2.5% monoolein plus 2.5% oleic acid were added to a diet containing 25% tristearin to facilitate formation of mixed micelles and thus increasing absorption of tristearin. No significant improvement in absorption of tristearin was observed. This is in accord with the results of Scribante and Favarger (10) who found no improvement in absorption of stearic acid when supplemented with triolein (67% stearic acid and 23% triolein) and fed at 15% of diet to rats. However, since supplementation of tristearin with oleic derivatives, in our experiments, caused an increase in total amount of fat absorbed, activity of alkaline phosphatase increased almost twofold.

Replacing 5% tripalmitin with 5% triolein in the third experiment increased both fat absorption and alkaline phosphatase activity.

These results confirm the previous reports that fatty meals increase the activity of total and "intestinal" alkaline phosphatases and extend this observation by showing that triolein is the fat presumably responsible for this increased activity.

Preliminary studies with 2 rats cannulated in the thoracic duct, showed that feeding 1 ml of corn oil by stomach tube to fasted rats increased total alkaline phosphatase concentration of lymph from 1.8 to 3.0 Sigma units and L-phenylalanine sensitive component of lymph alkaline phosphatase from 0.9 to 2.8 after 2 hr. Continuous drainage of lymph for 4 hr, however, caused the activity to drop to 2.3 for total and 2.2 for the L-phenylalanine-sensitive component. Most of the activity induced by feeding corn oil was intestinal in origin, suggesting a quantitative relationship between the absorption of fat and this enzyme. These observations agree with the previous findings in rat (1, 2) and man (3). Keiding (3) demonstrated that the electrophoretic mobility of alkaline phosphatase of human lymph is identical to that of intestinal fluid enzyme but different from that of peripheral blood. He concluded that intestinal alkaline phosphatase is supplied to lymph and this in turn is delivered to plasma, perhaps after being transformed in the liver.

Since the lymph alkaline phosphatase activity rose within 2 hr after feeding corn oil, the increase in activity may be as a result of increased rate of discharge of this enzyme from the mucosal cells rather than due to an increase in its production by these cells.

Whether the elevation in serum alkaline phosphatase in the presence of a high amount of absorbable fats is due to an increased requirement for this enzyme in the process of resynthesis of triglycerides in the intestinal mucosa, to the role of this enzyme in the transport of micelles through the intestinal epithelial cells, to an increase in secretion of bile salts as a result of stimulation by ingestion of a high amount of fat, or to other factors cannot be determined from these studies.

Table V reveals that different dietary

TABLE V. Effect of Calcium, Phosphorus, and Calcium/Phosphorus Ratio on Serum Alkaline Phosphatase.

Treatment			Serum alkaline phosphatase[a]		
Ca (mg/g of diet)	P (mg/g of diet)	Ca/P	Total (Sigma units)	Intestinal (Sigma units)	% Intestinal
0.85	1.50	0.57	10.55 ± 4.20	5.85 ± 2.53	54.93
0.85	16.75	0.05	13.23 ± 5.43	6.76 ± 3.29	51.09
5.00	5.85	0.85	7.51 ± 2.42	4.27 ± 1.71	56.86
20.62	1.50	13.75	9.09 ± 2.30	4.19 ± 2.78	46.09
20.62	5.85	3.52	10.95 ± 5.63	6.16 ± 3.49	56.26
20.62	16.75	1.23	8.30 ± 1.80	4.57 ± 1.37	55.06

[a] Average of 30 rats ± SE of mean; differences not statistically significant.

levels of calcium or phosphorus did not alter the concentration of alkaline phosphatase significantly. This does not agree with the findings in sheep (4) and poultry (5) in which low calcium diets caused an elevation in the activity of this enzyme. Whether the discrepancy is due to species variation, difference in assay procedure, duration of low calcium diets, or result of other constituents of diet is not clear. Since alkaline phosphatase activity increases in some disorders of bone, the dietary levels of calcium and phosphorus may influence the concentration of this enzyme in such disorders.

Summary. Increased fat asborption led to increased lymph, total serum, and L-phenylalanine-sensitive "intestinal" alkaline phosphatases activities. Triolein, which is almost completely absorbed, increased the activity of this enzyme, whether ftd alone or in combination with tristearin or tripalmitin. Dietary levels of calcium or phosphorus had no effect on the total serum or "intestinal" alkaline phosphatase activities of normal, growing rats.

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