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Relationship of Blood Concentrations of Calcium, Phosphate, Gastrin and Calcitonin to the Onset of Feeding in the Rat[1] (38913)

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Recently Milhaud and associates (1–3) and Talmage *et al.* (4) reported that in rats maintained on a rigorously regulated feeding schedule, plasma calcium and phosphate concentrations fluctuated in a daily rhythmic manner related primarily to the start of the feeding period. Both plasma calcium and phosphate levels fell prior to the onset of feeding and remained low during the first few hours of the normal feeding period even if food was withheld. Plasma calcium concentrations rose prior to or during the first part of the nonfed (fasting) period but were maintained at a constant level throughout the greater part of this period. However, a continuously rising plasma phosphate concentration characteristically occurred during the nonfed period.

Extending these initial observations, Talmage *et al.* (4) reported that, in rats in which ^{45}Ca and ^{32}P had been administered for 5 days or more before study, the daily plasma concentrations of these radioisotopes also changed like their stable counterparts except to a much greater degree. The only difference noted was in rats in which the nonfed period was extended past the scheduled time of feeding. Under these conditions, both plasma calcium and phosphate concentrations followed the pattern of the fed rats, while ^{45}Ca and ^{32}P concentrations in the nonfed rats rose instead of falling.

The studies reported here delineate further the changes occurring immediately before and after the time at which food is offered. Changes in plasma calcium, phosphate and their respective radionuclides were compared to changes in the quantities of circulating gastrin and calcitonin determined by radioimmunoassay. Levels of circulating gastrin and calcitonin were evaluated because calcitonin is known to be a potent hypocalcemic, hypophosphatemic hormone in the rat (5), and gastrin is known to be a calcitonin secretagogue in several species (6, 7).

Materials and Methods. Male Holtzman rats, weighing 175–200 g, were adapted to a regular feeding cycle in which food was available for 12 hr and then withheld for 12 hr. The times of feeding and of food removal were maintained constant (±5 min) each day; the light period was also of 12 hr duration, half the rats being provided food during the "light" period and half during the "dark" period. All data were pooled relative to the start of the feeding period. The food was standard Purina Laboratory Chow containing 1.2% calcium and 0.85% phosphorous. Rats were acclimated to the feeding schedule for 3 wk before blood samples were obtained from the tail. With this schedule, the rats ate voraciously when food was placed in the cages, and they consumed approximately 20 mg food/g BW during the first hour of the feeding period.

Radioisotopes (100 μCi each of ^{45}Ca and ^{32}P) were administered one week before blood samples were obtained. The period studied in detail was from 4 hr prior to feeding through 2 hr after the onset of feeding. All radioisotope values were normalized to the −4 hr value obtained the same day. No more than one blood sample was obtained from each rat during a single feeding period, and usually 48 hr separated two blood collections from any one rat. Radioisotope concentrations of ^{32}P and ^{45}Ca were measured by liquid scintillation spectrometry as described earlier (4). Plasma phosphate was determined colorimetrically (8)

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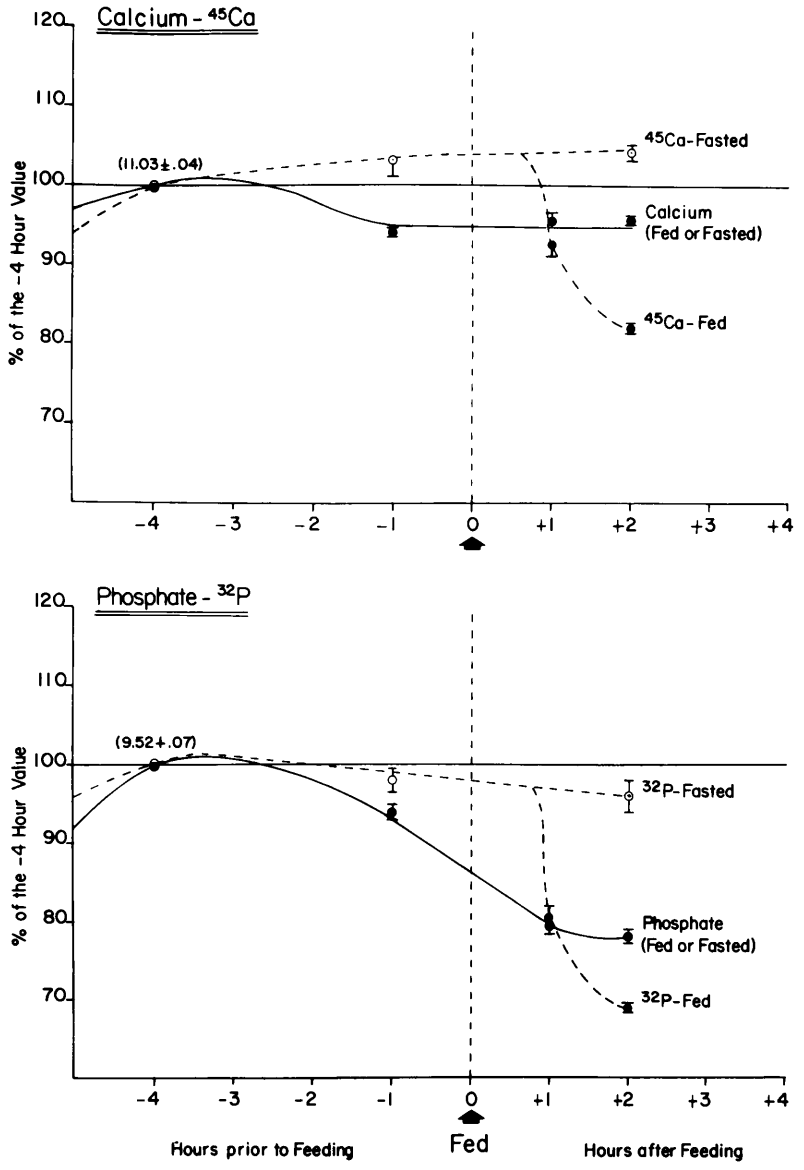

FIG. 1. Plasma Calcium and Phosphate Concentrations as Influenced by the Availability of Food. *Notes:* 1) Number in ( ) = concentration of stable ion in plasma 4 hr prior to time of feeding. 2) All points on graphs are means ± SE of 15–60 plasma samples. 3) Food available 12 hr each day. 4) Fasted = not fed at regular feeding time.

and calcium by automatic fluorometric titration with EGTA as modified from Borle and Briggs (9).

Concentrations of gastrin and calcitonin in serum were determined using radioimmunoassay procedures described previously in detail (6, 10–12). For analysis, sera were quickly obtained by centrifugation of collected blood samples and kept frozen at −20° until assayed. Radioimmunoassays for gastrin were conducted under "equilibrium" conditions using guinea pig antiserum to porcine gastrin at a final dilution of 1:100,000. Synthetic 1–17 human gastrin (ICI, England) was used for both iodination with ^{125}I and as unlabeled reference

standard. Therefore, the levels of gastrin reported here for rat serum are in terms of pg-equivalents of synthetic human gastrin. Bound and free labeled gastrin were separated using amberlite resin (10, 11). Radioimmunoassays for calcitonin were conducted under "nonequilibrium" conditions using chicken antiserum to rat calcitonin at a final dilution of 1:2000. Highly purified rat calcitonin (12) was used for both iodination with ^{125}I and as unlabeled reference standard. Bound and free labeled calcitonin were separated using dextran-coated charcoal (13). For both radioimmunoassays, serum samples were tested in duplicate or triplicate (usually in two or more separate assays) at final serum concentrations not exceeding 10% (v/v). Bound and free labeled fractions both were counted by automated solid scintillation spectrometry. For both immunoassays, intra- and interassay variations, as reported previously (6), did not exceed 15% and 20%, respectively. The lower limits of detectability of gastrin and calcitonin in serum were 20–30 pg/ml and 120–240 pg/ml, respectively.

Results. Changes in plasma calcium, phosphate and their respective radionucleide counterparts are presented in Fig. 1. Plasma calcium concentrations fell during the 2 hr period before feeding and remained low through the 2 hr postfeeding period studied regardless of whether or not the rats were fed at the scheduled time. Radiocalcium values rose during the prefeeding period but fell abruptly by the first hour after feeding. If the rats were fasted instead of fed, ^{45}Ca did not fall but rather continued to rise.

The phosphate and ^{32}P changes closely resembled those for calcium and ^{45}Ca, respectively. Plasma phosphate concentrations continued to fall during the first 2 hr after the onset of the feeding period in both fed and fasted rats. ^{32}P concentrations fell abruptly after feeding.

Plasma levels of gastrin and calcitonin are shown in Fig. 2. Prior to the time of feeding, plasma gastrin concentrations declined rapidly. This trend was reversed rapidly following the start of the feeding cycle, blood gastrin levels rising approximately

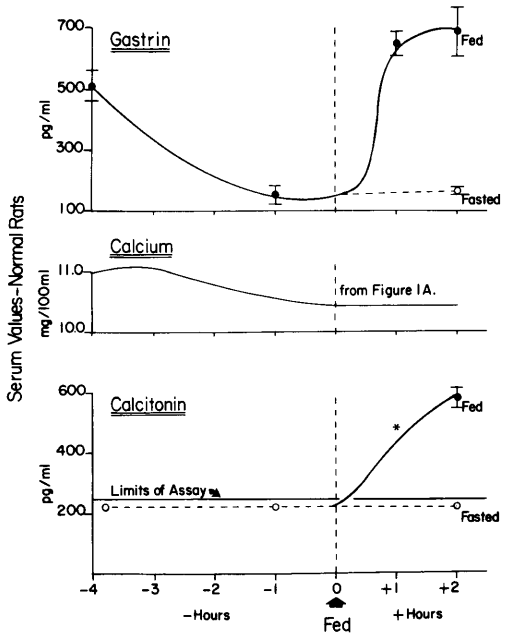

FIG. 2. Serum Gastrin and Calcitonin Concentrations as Influenced by the Availability of Food. *Notes:* 1) Points on graphs are means ± SE of serum samples taken from 12 to 16 rats (calcium values are for plasma). 2) Fasted = not fed at regular feeding time. 3) * —Calcitonin values at 1 hr after food became available ranged from nondetectable to > 600 pg/ml.

fourfold. If food was withheld, a low plasma level of gastrin was maintained.

Plasma calcitonin concentrations remained below the limits of assay sensitivity (<240 pg/ml) except immediately after feeding at which time they increased dramatically (a minimum of 2.5-fold). Plasma calcium concentrations superimposed on this graph illustrate that calcitonin was not detectable in the plasma of these rats even when the plasma calcium concentration was in the high normal range of 11 mg/100 ml, and that the dramatic increase observed in the concentration of calcitonin occurred during the time when plasma calcium concentrations actually were at their lowest, i.e., approximately 10.5 mg/100 ml.

There were no significant differences in the changes in plasma concentrations of either gastrin or calcitonin whether rats were offered food in the "dark" period or in the "light" period. It should also be noted that the onset of the rapid fall in

plasma ^{45}Ca and ^{32}P values appeared to coincide closely with the observed rapid rise in plasma gastrin and calcitonin concentrations.

Discussion. These studies confirm and extend earlier related observations by Milhaud and associates (1–3) and from our laboratory (4). There are several conclusions that can be reached. The first is that the daily fluctuations in plasma calcium and phosphate are related to the standardized feeding schedule and not the light–dark cycle, since varying the latter did not influence the patterns of response. The second point is that the stimulation of calcitonin secretion also was related to feeding and not to the existing plasma calcium level, since the marked increase in plasma calcitonin occurred after eating and at a time when plasma calcium was at its lowest observed concentrations. In fact, during the course of these experiments, the only time that calcitonin could be measured in plasma was shortly after animals began to eat. Plasma gastrin concentrations also rose following eating as anticipated. The surprising feature concerning gastrin was that in these trained rats, feeding produced plasma gastrin levels which appeared unusually high (i.e., ~700 pg/ml). Although in the pig and man, increased levels of blood gastrin can promote calcitonin secretion (6, 7), the cause and effect relationship between the release of one hormone and the release of the other cannot be determined in these experiments.

The cause of the fall in plasma calcium and phosphate prior to feeding was not elucidated other than that calcitonin did not appear to be responsible because plasma concentrations of calcitonin were lowest (i.e., unmeasurable) during this period and because plasma radionuclide concentrations (an index of bone metabolism) did not fall with their stable counterparts prior to feeding.

It is most interesting that a situation appears to exist in which plasma calcium and phosphate concentrations were influenced by intestinal-related stimuli to the extent that they actually fell to their lowest points during a time of active absorption of calcium and phosphate from the intestinal tract. The reasons for these paradoxical changes remain to be elucidated.

Summary. Daily fluctuations in plasma calcium concentrations in rats trained to a closely regulated feeding pattern have been compared to corresponding plasma gastrin and calcitonin concentrations. The time period studied was that extending from 4 hr prior to the start of the feeding period through the first 2 hr of feeding. Both plasma calcium and phosphate levels fell prior to the start of the feeding period and remained low at least for the first 2 hr of feeding. This pattern was also observed in rats in which food was withheld for 2 hr past the regular feeding time. Plasma ^{45}Ca and ^{32}P concentrations (radionuclide injected at least one week prior to sampling) did not follow the pattern of their stable counterparts. Instead, these values rose or remained constant until after feeding had commenced, after which they fell precipitously. Both plasma calcitonin and gastrin levels rose rapidly after the start of the feeding period. The primary point of emphasis is that calcitonin secretion was produced in these rats by an intestinal related stimulus and not by a rise in plasma calcium concentration.

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1. Milhaud, G., Perault-Staub, A. M., and Staub, J. F., J. Physiol. **222**, 559 (1972).
2. Perault-Staub, A. M., Staub, J. F., and Milhaud, G., Endocrinology **94**, 480 (1974).
3. Perault-Staub, A. M., Staub, J. F., and Milhaud, G., *in* "Calcium-Regulating Hormones" (R. V. Talmage, M. Owen, and J. A. Parsons, eds.), p. 229. Excerpta Medica, Amsterdam (1975).
4. Talmage, Roy V., Roycroft, J. H., and Anderson, J. J. B., Calc. Tiss. Res., **17**, 91 (1975).
5. Hirsch, P. F., and P. L. Munson, Physiol. Rev. **49**, 548 (1969).
6. Cooper, C. W., McGuigan, J. E., Schwesinger, W. H., Brubaker, R. L., and Munson, P. L., Endocrinology **95**, 302 (1974).
7. Hennessy, J. F., Wells, S. A., Jr., Ontjes, D. A., and Cooper, C. W., J. Clin. Endocrinol. Metabol. **39**, 487 (1974).
8. Chen, P. S., and Neuman, W. F., Amer. J. Physiol. **180**, 623 (1955).

9. Borle, A. B., and Briggs, F. N., Anal. Chem. **40,** 339 (1968).
10. McGuigan, J. E., Isaza, J., and Landor, J. H., Gastroenterology **61,** 659 (1971).
11. Yalow, R. S., and Berson, S. A., Gastroenterology **58,** 1 (1970).
12. Burford, H. J., Ontjes, D. A., Cooper, C. W., Parlow, A. F., and Hirsch, P. F., Endocrinology **96,** 340 (1975).
13. Herbert, V., Lau, K. S., Gottlieb, C. W., and Bleicher, S. J., J. Clin. Endocrinol. Metabol. **25,** 1375 (1965).

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