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Avian Pancreatic Polypeptide (APP) Levels in Fasted-Refed Chickens: Locus of Postprandial Trigger?[1] (41328)

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Abstract. Fasting and refeeding studies were carried out in young normal chickens (6–8 weeks old) in an effort to establish a correlation between movement of the digesta through the alimentary tract and perturbations in plasma insulin (IRI) and pancreatic polypeptide (APP) levels. In some experiments the gut was blocked by an exteriorized ligature to prevent movement of the digesta past that locus. Six different gut sites were selected for these refeed-blockade experiments. Progressive fasting of chicks for 24–96 hr decreased basal plasma APP levels to values 27.8–40.2% below fed control levels, was without effect on glucose levels, and either was without effect or slightly elevated basal IRI levels (96 hr only). Refeeding for 10 min after varying periods of fasting led to a rapid tripling of APP levels, little or no change in plasma glucose, and slight increases in IRI levels (except in the 96-hr fasted group). Chicks fasted 24 hr and then allowed to eat *ad libitum* up to 90 min were analyzed for plasma glucose, APP, IRI, and the gut examined for the distance of digesta movement through the GI tract. Measurements were made at seven times between 5 and 90 min postprandial. APP levels tripled before the first (5 min) sample; at this time the digesta had already passed through the duodenum and entered the ileum. A second "burst" of APP release appeared to occur at later times as the food particles traversed the small intestine. Insulin levels peaked at 10 min, a time at which the digesta reached the upper one-third of the ileum and plasma glucose rose to equal the prefast control level (215 mg/dl). Studies with ligature-blockades at different gut sites indicated that crop distention alone probably was responsible for the initial, rapid release of APP to the plasma. Gut distention was of little or no effect on any parameter. However, as the digesta moved further along the gut, additional release of APP was not observed until the digesta passed through the upper one-third of the ileum and was most prominent when food was prevented from passing the yolk stalk blockade. It is concluded that the two-phase release of APP in the postprandial state is due initially from neural activation emanating from the distended crop, and later from a probable humoral agent released from the upper ileum of the young chicken.

Pancreatic polypeptide, a recently discovered hormone-like peptide emanating from endocrine-type cells of the vertebrate pancreas, circulates at levels of 70–100 pg/ml plasma in man, 6–10 ng/ml in birds, and 110–130 pg/ml in dogs (1–5). Unlike the transitory increases in plasma glucose and insulin levels observed following each daily meal in man, HPP (human pancreatic polypeptide) levels rise quickly and remain significantly elevated for several hours after eating (e.g., 1, 6). During prolonged fasting (69–84 hr) in man, HPP levels fluctuate daily in such a pattern that a progressive increase is observed between 0990 and 2100 hr, only to decline overnight but to levels significantly higher than the plasma levels observed 24 hr earlier (1, 6). Overnight fasts in birds frequently decrease plasma APP (avian pancreatic polypeptide) levels by about 50% (3, 7). Refeeding mixed meals, or meals high in one of the major nutritive substrates, causes an immediate (1–3 min) rise in plasma PP levels in all nonavian vertebrates examined. Proteinaceous substrates appear most efficacious in elevating plasma PP during the refeeding response in man, birds, and dogs (1, 3, 8).

Evidence has accumulated in mammals indicating that both neural and hormonal "triggers" are responsible for the postprandial rise in plasma PP. Thus, in "chew and spit" studies in humans, as well as in studies where food is chewed and swallowed but diverted prior to entering the

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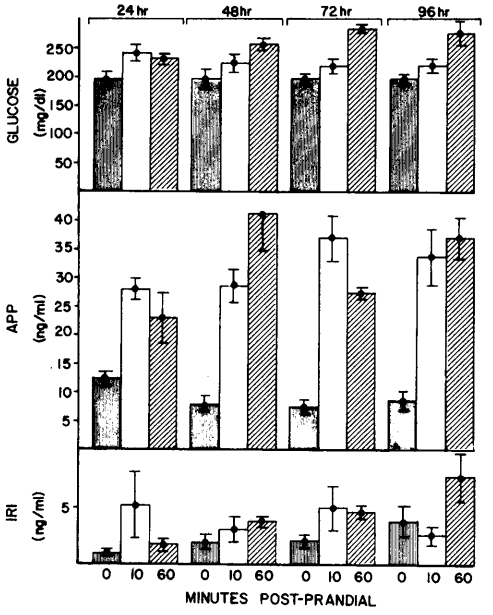

FIG. 1. Plasma alterations during progressive fasting and refeeding of chickens. Fasted (0 min) and two refed (10 and 60 min) plasma levels of glucose, APP, and IRI are shown for groups fasted 24, 48, 72, and 96 hr. Each group contained four chickens. The height of each column represents the mean; vertical lines are standard errors of the means. Fasted birds = vertical hatched bars; refed 10 min = open bars; and refed 60 min = diagonal hatched bars. Prefasting plasma levels for glucose, APP, and IRI were 215 ± 8 mg/dl, 14.5 ± 3.1 ng/ml, and 1.62 ng/ml, respectively for $N = 16$.

duodenum, an immediate elevation of HPP is observed (9, 10). However, the magnitude of the postprandial rise varies considerably, sometimes being equal to or, more commonly, is less than that observed when the digesta is allowed full alimentation (9, 11). Further, distention of the stomach or duodenum with isotonic saline increases HPP levels in conscious patients; again, the perturbation in HPP levels is far less than that observed with the digestion of a meal (11, 12). The vagus nerve appears to play a major role in PP release in response to the presence of food in the upper gut. However, the additional role of humoral "triggers" is suggested by the magnitude of HPP response observed as digesta proceed from the duodenum toward the colon as well as by the observation that the protracted postprandial response still obtains after truncal vagotomy (18).

Pancreatic polypeptide first was discovered in birds (13, 14) and the fasting-refeeding plasma response of APP in this animal class is well documented (3, 7, 14). However, the site/locus of those "triggers" for pancreatic release of APP in response to the presence of food in the alimentary tract is yet to be elucidated, as is the nature of the proposed "triggers." The purpose of this study was to identify those factors which contribute to the immediate postprandial APP response in young chickens.

Methods. *Animals.* DeKalb (Fig. 1) and single comb white leghorn–DeKalb cross chickens (Figs. 2, 3), 6–8 weeks old (600–850 g), were obtained from Rich-Glo Farms, El Campo, Texas, and housed (12 hr light followed by 12 hr dark) at least one week prior to use. Purina (Growena) feed and water were available *ad libitum.*

Progressive fasting–Refeeding study. Young chickens were sampled (cardiac puncture), then fasted 24, 48, 72, or 96 hr, sampled again, and then refed the usual chow. At 10 and 60 min after refeeding commenced additional blood samples were taken and analyzed for glucose, insulin (IRI), and pancreatic polypeptide (APP) levels.

Digesta movement vs. plasma APP levels. Blood samples were taken from fed birds which then were fasted exactly 24 hr; another blood sample then was obtained. The chickens then were allowed to refeed (normal feed which had been previously dyed with Evans Blue, T-1824) for 5, 10, 15, 30, 45, 60, or 90 min. Birds were sacrificed at each of the foregoing times, and the gut exposed and measured for the distance travelled by the food with T-1824 marker. Distance travelled from the inferior edge of the gizzard was noted relative to the leading edge of the marker, and percentage of total intestinal distance travelled also was recorded. Blood samples were analyzed for glucose, IRI, and APP.

Gut blockade experiments. Under sodium pentobarbital (Nembutal) anesthesia, grocery store-type string was placed loose-

ly around various levels of the alimentary tract of fed chickens ranging from the inferior margin of the crop to the level of the yolk stalk remnant of the distal small intestine. Care was taken to avoid enclosing known/visible vagal elements within the ligature. The ends of the loose string were exteriorized through the abdominal incision, the muscle layers and skin sutured, and the birds allowed to recover 48 hr during which time they were fasted. No impediment to passage of the normal food (eaten prior to surgery) occurred until the time of the experiment, at which time the strings were pulled tight in the unanesthetized birds, closing the prepositioned knot, and they once again were allowed to refeed the experimental dyed food. Previous control studies indicated that placement and/or tying of strings per se was without effect on plasma APP levels. After tying the strings all birds were allowed to eat *ad libitum* for 60 min, another blood sample was then taken, and then the birds were killed immediately. The gut was examined for presence of dyed digesta to make certain that despite 60 min time, food had not passed beyond the tightened knot. Blood samples were analyzed for glucose, IRI, and APP.

Blood analyses. Plasma glucose was measured by the Glucostat (oxidase, Worthington Biochem) method. Plasma was diluted 1:2 with buffer and insulin (IRI) was assayed in triplicate by the standard double-antibody method of Hales and Randle (15), using chicken insulin as standards, and guinea pig anti-chicken insulin and rabbit anti-guinea pig sera as the antibodies. Minimal detectable IRI in 12 assays was 6 pg/tube (P = 95%), intraassay coefficient of variation was 8.2 ± 1.0% and inter-assay coefficient of variation was 4.1%. Bound insulin was collected on cellulose acetate membrane filters (Oxoid "Nuflow," 0.45 μm × 25 mm, Oxoid Lt., England). Immunoreactive APP was measured in triplicate (on plasma previously diluted 1:6 with buffer) as described originally by Langslow *et al.* (14), employing chicken APP as the standard. The APP binding reagent consisted of specific antibodies to chicken APP raised in rabbits and the precipitating antibody was goat anti-rabbit serum. Separation of bound APP from free APP again was achieved by filtration using Oxoid filters, 0.45 μm × 25 mm. Sensitivity (P = 95%) of 12 APP assays was 13 pg/tube, the mean intraassay coefficient of variation was 12.2 ± 1.6% and the interassay coefficient of variation 12.6%. In our laboratory, filtration-separation of APP invariably yields higher values than those achieved by centrifugation methods. All data presented herein were collected by filtration methods. Both chicken hormone standards and specific antibodies were a generous gift of J. R. Kimmel, University of Kansas Medical School. APP levels presented herein are slightly higher than previously reported values and based upon our experience are probably due to the combination of the method of RIA, age of the bird, and the strain of chicken used. DeKalb breeds appear to have the highest basal plasma APP levels which we have encountered.

Statistical analysis. Student's t test (two-tailed) was employed; P values less than 0.05 were considered significant.

Results. *Progressive fasting–Refeeding study.* Plasma glucose, IRI, and APP levels obtained from chickens fasted 24–96 hr and then fed *ad libitum* are presented in Fig. 1. Generally, plasma glucose levels showed similar postprandial elevations in all fasted-refed groups, except that after 72 hr of fasting the 60-min refeeding levels were significantly ($P < 0.01$) higher than when chickens were refed after the 24-hr fasting period. Basal (time 0) IRI levels tended to rise over the 96-hr fasting period but these changes were not statistically significant. While various increases in plasma IRI were observed 10 and 60 min after refeeding, no definite pattern of IRI levels followed the plasma glucose excursions described. The only significant IRI change from the 0 level was in the 72-hr fasted group which had eaten for 60 min ($P < 0.02$). In contrast, postprandial levels of APP within each fasted group were significantly higher ($P < 0.001–0.05$) than the basal fasted (prefed) levels. However, the 10 and 60 min refed plasma APP levels did not differ significantly between groups. Progressive fasting,

without refeeding (time 0 samples), depressed APP levels by approximately 50–60%. From this study it was decided that in the absence of any required surgery a 24-hr fasting period was adequate for purposes of stabilization of metabolic parameters and induced the chicken to be more responsive to the early refeeding responses under study.

Digesta movement vs plasma APP levels. Birds were fasted 24 hr and then allowed to refeed (food contained a dye marker) for periods ranging from 5 to 90 min. Figure 2 presents the plasma glucose, APP, and IRI response as the recently acquired food moved down the gastrointestinal tract. The schematic diagram at the bottom of Fig. 2 allows one to estimate the position of the leading edge of the digesta relative to plasma hormone perturbations. Within 5 min of refeeding, food had passed the area of the gut associated with the entrance of the pancreatic ducts and had entered the "jejunum." At this time APP levels already had reached 20 ng/ml ($P < 0.001$), much faster than the increase in plasma glucose ($P < 0.001$) or the corresponding increase in plasma IRI ($P < 0.001$). Actually, IRI excursions were moderate, at best, despite the prolonged elevation in plasma glucose, the IRI levels peaking at 10 min, and returning to control levels by 45–60 min after feeding. Contrarily, APP levels rose approximately 400% within 5 min and 460% within 10 min of refeeding the previously fasted chickens. As indicated in Fig. 2, and quantified in Table I, digesta reached the yolk stalk region by 30 min and within an additional 60 min it had entered the cecal-colon region.

Gut blockade study. Chickens were surgically prepared with (untied) ligatures and then fasted 48 hr while recovering from surgical stress. The experiment commenced with tying of the ligatures to block the gut. As seen in Fig. 3, a modest elevation in plasma glucose appeared to occur the further down the tract the digesta was allowed to progress; no significant change in plasma IRI was noted. Unfortunately, gut distention could not be measured in this series. Food ingestion was not measured,

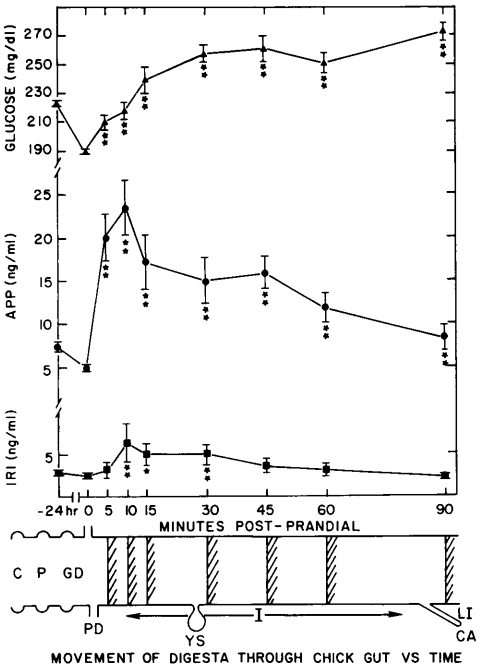

FIG. 2. Chicken plasma response to fasting and refeeding. Blood samples were taken on young chickens immediately before and immediately after a 24 hr fast, as well as at various times after *ad libitum* feeding as indicated, and analyzed for glucose, APP, and IRI. Birds were killed at each of the times indicated except at −24 hr and 0 times. All symbols represent the means; the vertical lines are standard errors of the means. The number of observations at each point ranged from 6 to 10. Significance: $*P < 0.01$, $**P < 0.001$ postprandial value significantly greater than fasting value. At the bottom of the figure is a schematic drawing of the chicken gut. The slanted lines (//////) indicate the leading edge of the dyed food at the postprandial times indicated. Legend: C, crop; P, proventriculus; G, gizzard; D, duodenum; PD, pancreatic duct; I, ileum (birds are not considered to possess a true jejunum (Refs. (16, 17)), YS, yolk stalk; CA, cecum; and LI, large intestine.

though all birds ate continuously for the first 20–25 min.

APP levels observed 60 min after refeeding in the patent (loose ligatures, untied) control birds approximated those seen at 60 min of refeeding in Fig. 1 (also 48 hr fasted). Pilot studies on fasted birds had shown that ligature tightening per se, regardless of position of the tie, was without effect on plasma APP levels during 60 min of observation. In those chickens with food

TABLE I. MOVEMENT OF DIGESTA THROUGH ALIMENTARY TRACT OF YOUNG CHICKENS UPON REFEEDING FOLLOWING A 24-hr FAST[a]

No. Observations	Time (min)	Distance from gizzard (cm)	Percentage of total gut	Approximate site of leading edge
5	5	29.0	28.6	Between duodenum and yolk stalk —22%
5	10	41.1	39.1	Between duodenum and yolk stalk —66%
2	15	35.0	45.4	Between duodenum and yolk stalk —64%
2	20	54.8	55.8	Between duodenum and yolk stalk —79%
3	30	55.6	66.9	Between yolk stalk and large intestine—22%
2	45	69.0	74.2	Between yolk stalk and large intestine—30%
2	60	68.0	79.5	Between yolk stalk and large intestine—47%
1	80	103.0	100.0	To large intestine
2	90	80.8	92.6	Between yolk stalk and large intestine—93%

[a] Also see Fig. 2, bottom schematic.

expanding the crop, but not progressing to the level of the proventriculus, a significant increase in plasma APP occurred (Fig. 3). Ligatures placed at the inferior margins of the proventriculus or the gizzard (see inset at top of Fig. 3) prior to refeeding did not alter APP levels from the fasting, control level. Limited absorption from these gut areas (16) evidently did not favor release of the polypeptide. When food/digesta was allowed to progress to the midduodenal level (midloop region, above entrance of bile and pancreatic ducts) a modest increase in plasma APP occurred. Crop distention was not obvious in any of these birds, all of whom ate "ravenously." The release of polypeptide in birds with ligatures at the entrance of the pancreatic ducts approximated that observed in birds with midduodenal blockades. The lack of APP response observed with blockades between the crop and midduodenal sites may indicate a high magnitude "trigger" response emanating from a region distal to the entrance of the ducts. The greatest APP response to refeeding was observed in those birds in which food was allowed to pass, unobstructed, as far as the yolk sac remnant (Fig. 3). Such a response, in the presumed absence of significant crop distention, may indicate functional involvement of the upper half of the avian ileum in the APP-refeeding response. APP release from the yolk stalk-ligated birds was double (25.8 vs 12.2 ng/ml) that observed in refed birds which had ligatures placed at the lower rim of the crop ($P < 0.03$).

Discussion. Progressive fasting of young chickens was without effect on control plasma glucose levels, a feature observed by many workers. Control plasma IRI levels also were stable for the first 72 hr, though the 96-hr values differed significantly ($P < 0.02$) from the 24-hr control level.

Major among the objectives of this study was to isolate what area(s) of the avian gut is responsible for the immediate and prolonged elevation in plasma APP observed after refeeding. Recent pilot studies (in two separate laboratories) have been carried out to distinguish between distension and absorption-secretion as contributing factors for the release of APP in response to a meal. Isolated gut segments (five different areas) of 9 cm each were prepared, cut at each end, and intubated with flexible plastic tubing; attached nerves and vessels were

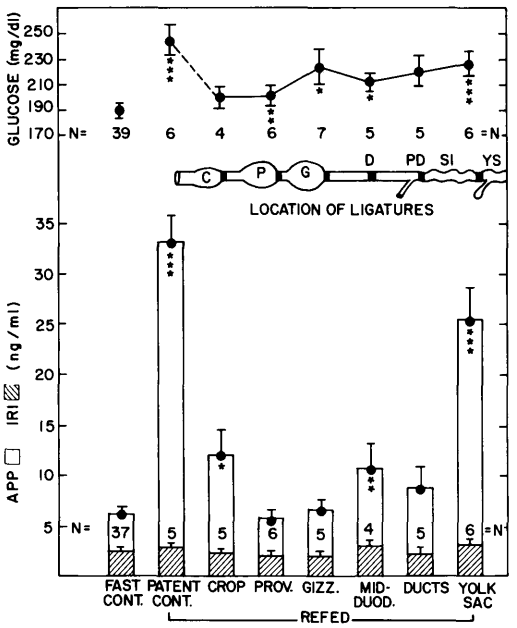

FIG. 3. Effects at 60 min of refeeding on plasma APP, IRI, and glucose levels in gut-blocked chickens. CONT, controls. The first group (fasted controls, CONT) represents all birds after the surgical ligature placement *and* after a 48-hr fast, but before feeding. The second group (patent controls) are birds with ligatures in place but remaining loose for refeeding. The other six groups represent gut blockade at different locations as indicated on the gut schematic at the top of the figure. The height of the entire column represents the plasma APP levels; the shaded portion represents the IRI levels in each case. Vertical lines are standard errors of the means. Corresponding fasting and 60-min refed glucose levels are shown above the bars. The number of observations is indicated by "N" for each parameter of each group. The linear (schematic) gut diagram is included to aid location of the placement of the gut blockades. None of the 60-min IRI values were significantly different from their respective fasting values ($P > 0.05$). SI, small intestine. Significance: $*P < 0.05$, $**P < 0.025$, $***P < 0.001$ for 60-min levels when compared with respective group fasting levels.

kept intact. Sacs were filled either with warm saline, or equal volumes of digesta obtained from the identical gut region of a refed (donor) bird. Thus, distention effects were attenuated and/or equalized in each of control (saline) and experimental (digesta) birds. The crop cannot be prepared as a gut sac but one may cannulate the orad portion and ligate the inferior margin so that graded volume distention may be evaluated as a factor in "triggering" APP release. Blood samples were taken at 1, 3, 5, 15, 30, and 60 min.

Results obtained from these pilot studies as well as those of J. R. Kimmel (personal communication) have been meaningful for the crop, less so for the gut sac preparations. Thus, degree of volume distention appears to be related to the immediate release of APP both as to the onset of release and to the relative magnitude of APP increment. Texture of crop digesta material also probably plays a role (feed is better than Bio-gel, etc.).

Gut sacs prepared from different areas of the duodenum-intestine have, when loaded with donor digesta, not led to consistent results when one assays plasma for APP. At best and suggestive only, the first segment of the ileum (birds are not considered to possess a true "jejunum," the ileum starting with that area associated with the papilla of Vater) is an area which needs closer scrutiny as one which may secrete a PP secretagogue.

The mere sight of food is not sufficient to increase APP levels, as Kimmel (3) demonstrated when he allowed previously fasted chicks to look at food but at the same time prevented prehension. While magnitude of crop distention (pilot studies cited above) generally was related to the magnitude of APP released, the crop can be distended to an even greater degree (two- to four-fold) by use of semidry food particles or nonnutritive expanders. Still, this nonabsorptive organ (16, 17) when greatly distended does not release APP to the magnitude seen when birds are allowed to feed *ad libitum* (Kimmel and Pollock, personal communication) and with the gut either unligated or ligated at the level of the yolk stalk remnant (present studies). Birds with crop ties ate surprisingly well, in fact they ate during most of the 60-min observation period. Obvious crop distention occurred, distention (qualitatively) much greater than that seen in the pilot studies or in birds ligated lower in the alimentary tract. Despite the much greater crop distention plasma APP levels rarely increased more than twofold,

while in unobstructed preparations or in yolk sac preparations APP levels increased fivefold above fasting control levels. Thus, Figs. 2 and 3, as well as the crop sac pilot study observations indicate collectively that neural elements, probably activated by crop distention, initiate the very early rise in chicken plasma APP observed in the first few minutes after commencement of meal eating. Atropine premedication or truncal vagotomy abolish the early postprandial rise in HPP in human ulcer patients (18). Pentobarbital has the same effect in young chickens. (3).

For total release of APP to occur in response to feeding, food/digesta must pass the avian duodenum and at least reach the first few centimeters of the ileum (see note above about the term "jejunum"). Thus, crop distention may initiate a burst of APP release but intestinal involvement is required (even after cessation of eating) to prolong secretion of APP an additional 75 min (Fig. 2). This postulate is supported by the data presented in both Figs. 2 and 3 where the presence of food at this level or beyond prolonged the APP release response (Fig. 2) although crop distention may not have occurred due to site of the blockade. The greatest APP response was observed in those birds with unobstructed guts to the level of the yolk stalk, suggesting that the upper half of the ileum in some way contributes to the "trigger effect" of the midduodenum region. *In vitro* gut sac preparation studies (see above) indicate that this response is more likely secretagogic than distention in nature. (It should be noted that fasted birds do not "crop regulate" food passage, thus distention of this structure would not necessarily be expected to be seen in Fig. 3, middle blockades (16, 19, 20)). The absorption of glucose would not appear to be important to this response (Fig. 2 and Ref. (21)) and other workers have ruled out the contribution of acid secretion (3, 10). Possibly the lipid content of a meal is important in this response (3) but based on studies on pigs, dogs, and man (e.g. 1, 3, 6, 10) it appears more likely that the presence of protein may indeed release a gut secretagogue which in turn provokes PP release from the pancreas. Intravenous pentagastrin and secretin have been demonstrated to be effective in mammals in this regard (1–3), and pentagastrin is a powerful *in vivo* (but not *in vitro*) secretagogue to APP release in chickens (21), while having no simultaneous effect on insulin or glucagon release. The role of the vagus nerve in such a secretagogic action in birds is yet to be evaluated although atropine and sodium pentobarbital are known to obtund postprandial effects (3). They also depress alimentation in birds.

In conclusion, progressive fasting decreases APP levels in chickens in contrast to the gradual elevation such a regimen has on HPP levels in humans. Meal eating elevates APP levels within 1–2 min in chickens and in general produces a biphasic APP response which lasts several hours. The initial APP response is probably due to afferent fibers (vagal) responding to crop distention. However, full expression of the postprandial APP response is not observed unless food/digesta passes out of the duodenum and enters the ileum. It is suggested that absorption of nutrients is less important in provoking APP release than that of release of a secretagogue from the ileum to activate pancreatic PP cells in the chicken.

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