Gastric Inhibitory Polypeptide

Its Physiologic Release and Insulinotropic Action in the Dog

Raymond A. Pederson, Ph.D., Harold E. Schubert, M.Sc., and John C. Brown, Ph.D., Vancouver, British Columbia, Canada

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SUMMARY

Studies were carried out in conscious dogs in which the immunoreactive gastric inhibitory polypeptide (IR-GIP) response to graded doses of oral fat (triglycerides) and glucose was investigated. The IR-GIP response to the doses of triglycerides used was greater and more prolonged than the response to the glucose loads employed. In addition, the relative insulinotropic potencies of exogenous porcine GIP and IR-GIP released by fat as against those released by oral glucose were assessed. When glucose was administered by the oral route, the immunoreactive insulin (IRI) response was magnified above the IRI response to a comparable intravenous glucose load. The serum IRI response to oral glucose was accompanied by a concomitant rise in serum IR-GIP levels, suggesting a causal relationship. IR-GIP released by oral fat was shown to augment the IRI response to an intravenous glucose load, resulting in an improvement of glucose tolerance. Fat-released IR-GIP augmented IRI levels to a lesser degree than either oral glucose or an infusion of porcine GIP. DIABETES *24*:1050-56, December, 1975.

Oral or intraduodenal administration of glucose results in a considerably greater rise in serum insulin levels than intravenous administration of glucose at the same rate.[1] This phenomenon has been attributed to the operation of an enteroinsular axis entailing release of insulin by a gut hormone. A search for the active agent(s) operating in this hormonal reflex has included investigation of the established gastrointestinal hormones, cholecystokinin-pancreozymin (CCK-PZ), gastrin, and secretin, as well as several impure duodenal and jejunal extracts.[2-5]

Criteria by which the role of a peptide in the enteroinsular axis is established must include demonstration that the peptide is released by oral secretagogues that normally release insulin (notably glucose) and demonstration that administration of exogenous peptide, in amounts mimicking its physiologic release, will augment the insulin response to elevated serum glucose. The established gastrointestinal hormones have failed to meet one or both of these criteria.

Evidence has been rapidly accumulating that suggests a role for gastric inhibitory polypeptide (GIP) in the enteroinsular axis. Dupre et al.[6] found that intravenous administration of porcine GIP would magnify the release of immunoreactive insulin (IRI) in response to an intravenous glucose load in man with attendant improvement in glucose tolerance. No effect on IRI secretion was observed when GIP was infused at the same rate under fasting conditions. A dose-response relationship between intravenously administered GIP and IRI release was demonstrated in the fasted dog by Pederson et al.[7] Comparison of serum levels of IR-GIP observed in these studies with those of Dupre suggested that at low serum GIP concentrations, exogenous GIP potentiates the insulin response to glucose, whereas at higher levels it may stimulate insulin release independently.

The development of a specific and sensitive radioimmunoassay for GIP[8] provided a means for studying circulating levels of immunoreactive GIP (IR-GIP). Ingestion of glucose has been shown to be a potent stimulus for IR-GIP release in man[9] and, when taken together with the evidence of insulin release by pure GIP, supports a role for GIP as an active principle in the enteroinsular axis. IR-GIP levels were also found to be elevated following ingestion of fat in

From the Department of Physiology, University of British Columbia, Vancouver, British Columbia, Canada.

Address reprint requests to Dr. R. A. Pederson, Department of Physiology, University of British Columbia, Vancouver, B. C., Canada V6T 1W5.

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man,[10] with results indicating different patterns of IR-GIP release associated with ingestion of fat and glucose. The release of IR-GIP by fat is more consistent with the known enterogastrone activity of GIP[11] than with insulin release from the pancreas.

The aims of the present study, based on the uncertainties regarding physiologic release and action of GIP, are twofold—to quantitatively characterize the IR-GIP response to graded doses of oral fat and glucose and to assess the relative insulinotropic potencies of exogenous porcine GIP and IR-GIP released by fat as compared with oral glucose.

MATERIALS AND METHODS

Six dogs of both sexes weighing 23 to 28 kg. were used in this study. All experiments were performed in conscious dogs previously fasted for eighteen hours. Blood was collected through an indwelling catheter in the right or left cephalic vein. Serum was aliquoted for measurement of serum glucose, immunoreactive insulin, and immunoreactive GIP. Glucose was administered orally as a 20 per cent solution in distilled water. The source of fat used was Lipomul (Upjohn) a palatable emulsion containing 66 gm. of triglycerides per 100 ml. Distilled water was administered orally in control experiments. Triglycerides, glucose, or water were administered orally to dogs by a glass syringe fitted to 30 cm. of 0.2-cm. (inside diameter) polyethylene tubing. The end of the tube was held inside the cheek near the posterior molars. Liquid deposited here induced swallowing. Intravenous GIP and glucose were administered in saline and water solutions, respectively, via indwelling intravenous catheters connected to a Harvard infusion pump.

Assays

Serum glucose concentrations were determined in duplicate by a Beckman Glucose Analyzer.

Serum IRI concentrations were determined in duplicate by the Phadebas Insulin Test (Pharmacia AB, Uppsala, Sweden).

Serum IR-GIP concentrations were determined in duplicate by the radioimmunoassay described by Kuzio et al.[8] Serum IR-GIP concentrations less than 0.1 ng. per milliliter could not be reliably detected in this assay and are noted as not detectable (N.D.).

Analysis of Data

Results are expressed as mean plus or minus standard error.

Unless otherwise indicated, serum IRI and glucose values are expressed in terms of change from the mean IRI and glucose concentrations measured in three fasting blood samples taken at fifteen-minute intervals prior to the start of the experiment.

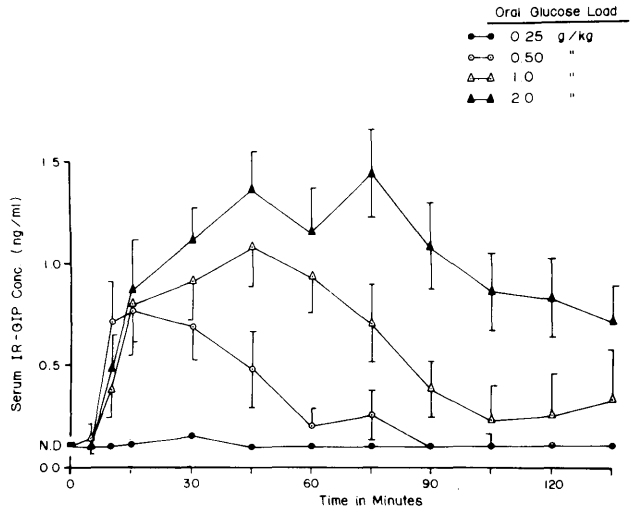

FIG. 1. IR-GIP responses following ingestion of 0.25, 0.5, 1.0, and 2.0 gm. per kilogram glucose.

RESULTS

Release of IR-GIP by Oral Glucose

In thirty-two experiments on four dogs, glucose was administered orally at 0.25, 0.5, 1.0, and 2.0 gm. per kilogram body weight (eight experiments at each dose level). Distilled water (100 ml.) was administered in eight control experiments. Figure 1 shows the serum IR-GIP responses to the four doses of oral glucose. In control experiments, serum IR-GIP levels showed no change from fasting levels (N.D. to 0.2 ng. per milliliter). With an oral glucose load of 0.25 gm. per kilogram, no significant increase in serum IR-GIP concentration over fasting levels was observed. Following a dose of 2 gm. per kilogram, circulating levels of IR-GIP rose to a peak of 1.4 ± 0.2 ng. per milliliter seventy-five minutes after ingestion of glucose.

Comparison of Serum IRI Responses to Oral and Intravenous Glucose

The IRI response to a one-hour intravenous infusion of 0.6 gm. per kilogram glucose was compared with the response to an oral glucose load of 1.0 gm. per kilogram (figure 2C). Six experiments of each type were performed on three dogs. The IRI response to oral glucose was considerably greater than to intravenous glucose (figure 2B), with a 100 per cent difference

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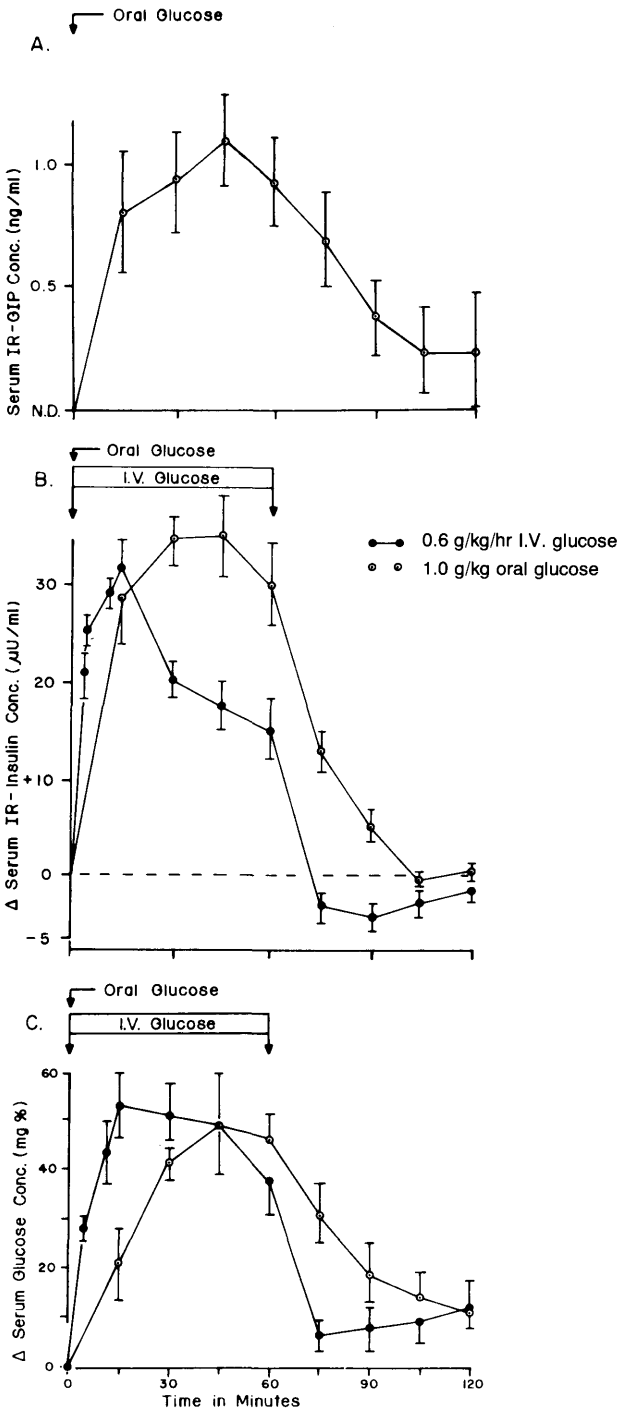

FIG. 2. Comparison of the changes in serum (A) immunoreactive GIP, (B) immunoreactive insulin, and (C) glucose concentrations in response to 1.0 gm. per kilogram oral glucose and 0.6 gm. per kilogram per hour intravenous glucose.

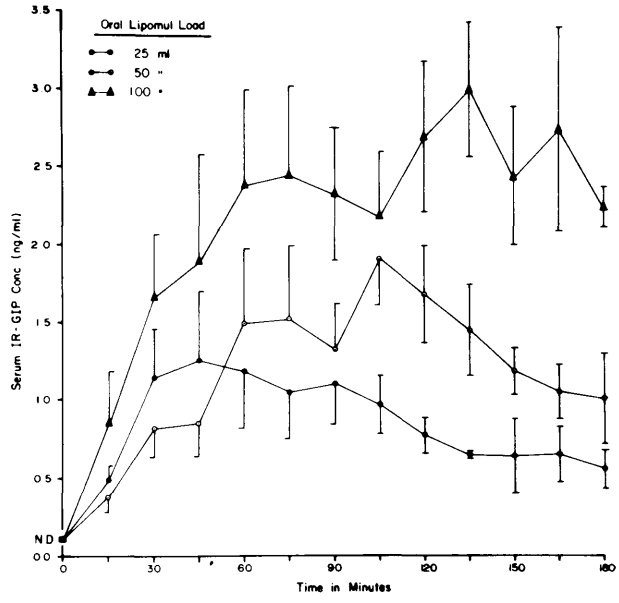

FIG. 3. IR-GIP responses to ingestion of 25, 50, and 100 ml. Lipomul (triglycerides).

in mean incremental IRI values at the forty-five-minute period ($p < 0.005$). It is important to this comparison that during the periods of maximum difference in incremental IRI (interval thirty through sixty minutes), the incremental serum glucose values in the two sets of experiments were not significantly different (thirty minutes $p > 0.05$, forty-five minutes $p > 0.40$, sixty minutes $p > 0.10$). The rise in serum IR-GIP levels following oral glucose (figure 2A) was simultaneous with the increase in IRI, with peaks in both responses occurring at forty-five minutes. Intravenous glucose produced no changes in fasting IR-GIP levels.

Release of IR-GIP by Oral Triglycerides

Figure 3 shows the IR-GIP response to ingestion of 25, 50, and 100 ml. of Lipomul (16.5, 33, and 66 gm. triglycerides, respectively) in twenty-four experiments on four dogs (eight at each dose level). In eight control experiments, dogs received 100 ml. of water orally. The peak value of serum IR-GIP achieved with the highest dose of triglycerides was 2.99 ± 0.43 ng. per milliliter at the 135-minute period. In four experiments it was found that after 100 ml. oral Lipomul, four and one-half to five hours was required for IR-GIP levels to return to fasting values.

Serum IRI and glucose concentrations were measured in all the oral-triglyceride experiments. No significant changes from control values in serum IRI or

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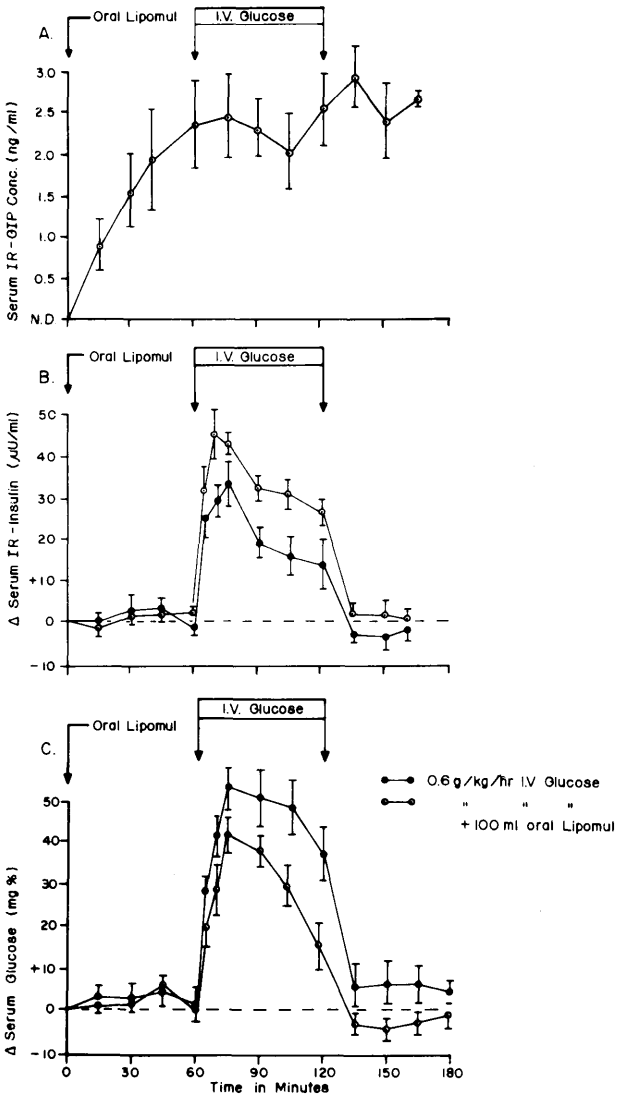

FIG. 4. The effect of 100 ml. oral Lipomul on incremental serum concentrations of (B) IRI and (C) glucose resulting from a one-hour intravenous infusion of 0.6 gm. per kilogram glucose. Figure 4A shows the serum IR-GIP response in experiments in which 100 ml. oral Lipomul was administered.

glucose concentrations were observed.

Effect of Oral Triglycerides on Serum IRI and Glucose Responses to an Intravenous Glucose Load

Oral administration of 100 ml. Lipomul was performed in six experiments on four dogs and of 100 ml. of water in the same number of control experiments. A one-hour glucose infusion (0.6 gm. per kilogram) was initiated sixty minutes following Lipomul or water ingestion, so as to elevate circulating glucose levels during a time when serum IR-GIP levels were rising to maximum in the Lipomul experiments (figure 4A). Figure 4B shows that the IRI response to intravenous glucose was greater following oral Lipomul than in control experiments. Concomitantly with the greater incremental IRI response, the toler-

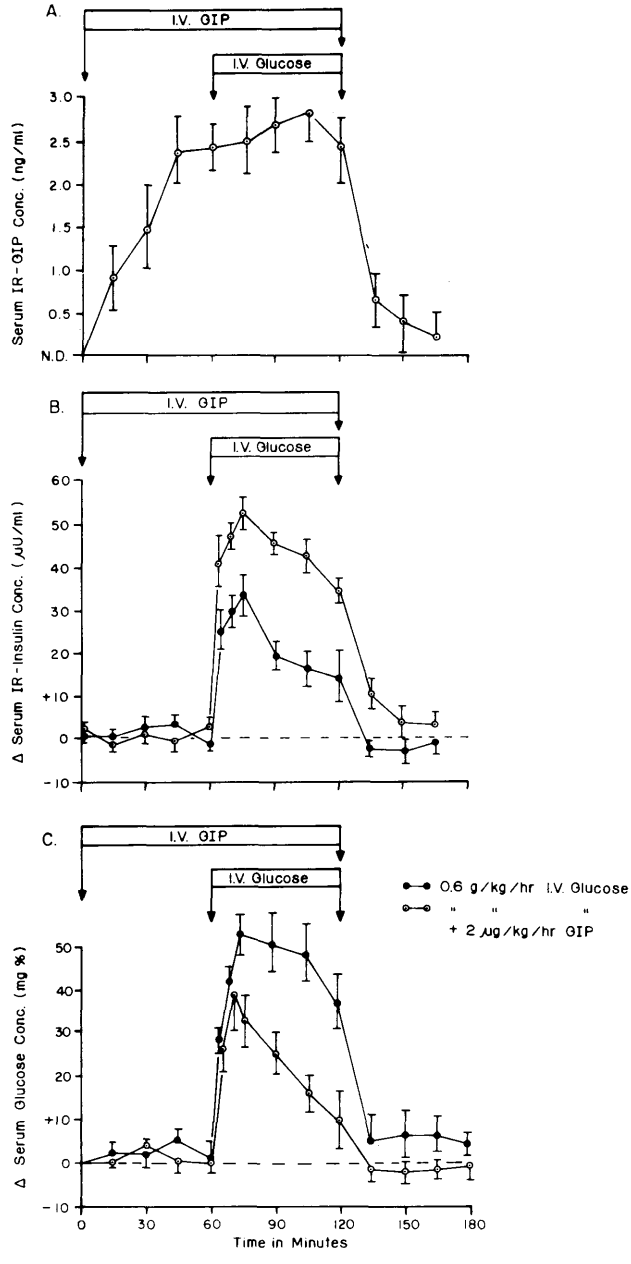

FIG. 5. The effect of a two-hour intravenous infusion of 2.0 μg. per kilogram per hour of GIP on serum concentrations of (B) IRI and (C) glucose resulting from a one-hour intravenous infusion of 0.6 gm. per kilogram glucose. Figure 5A shows the serum IR-GIP levels achieved during the GIP infusion.

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ance to intravenous glucose was improved after oral triglycerides (figure 4C). At the period of maximum difference (105 min.), the mean incremental serum glucose value was 37.5 per cent less than in control experiments ($p < 0.05$).

Effect of Exogenous GIP on Serum IRI and Glucose Response to Intravenous Glucose

In six experiments on three dogs, exogenous porcine GIP was given as an intravenous infusion of 2.0 μg. per kilogram per hour for two hours to achieve circulating levels of IR-GIP (figure 5A) comparable to the response produced by 100 ml. oral Lipomul (figure 4A). In six control experiments, saline was infused. Figure 5B shows that the serum IRI response to intravenous glucose was greater during intravenous GIP infusion than in control experiments. The tolerance of intravenous glucose was improved when GIP was infused (figure 5B). At the 105-min. period the mean incremental serum glucose value was 64.6 per cent less than the corresponding control value ($p < 0.01$).

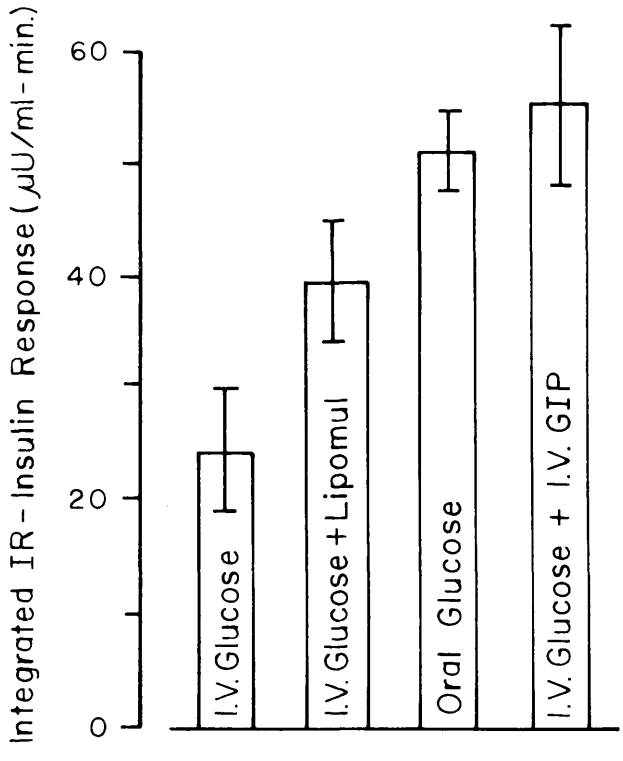

FIG. 6. The integrated IRI responses to 0.6 gm. per kilogram per hour intravenous glucose, 0.6 gm. per kilogram per hour intravenous glucose plus 100 ml. oral Lipomul, 1.0 gm. per kilogram oral glucose and 0.6 gm. per kilogram per hour intravenous glucose plus two-hour intravenous infusion of 2.0 μg. per kilogram per hour GIP.

Comparison of the Integrated IRI Responses to Oral Glucose, Intravenous Glucose, Intravenous Glucose plus Oral Triglycerides, and Intravenous Glucose plus Intravenous GIP

Figure 6 shows the integrated incremental IRI responses following: (1) oral glucose, 1.0 gm. per kilogram (figure 2B); (2) intravenous glucose, 0.6 gm. per kilogram per hour (figures 2B, 4B, and 5B); (3) intravenous glucose (0.6 gm. per kilogram per hour) plus 100 ml. oral Lipomul (figure 4B); (4) intravenous glucose (0.6 gm. per kilogram per hour) plus intravenous GIP (2.0 μg. per kilogram per hour) (figure 5B).

In all experimental procedures where serum IR-GIP levels were elevated, integrated IRI responses were significantly greater than the response to intravenous glucose (oral glucose $p < 0.005$, oral triglycerides $p < 0.05$, and intravenous GIP $p < 0.005$). These results show that:

1. The IRI response to oral glucose was more than 100 per cent greater than the response to intravenous glucose even though serum glucose curves were not significantly different during the interval of maximum difference in circulating IRI levels.
2. When intravenous glucose was preceded by oral triglycerides, the IRI response was significantly less than when intravenous GIP was administered ($p < 0.05$), despite comparable circulating IR-GIP levels.
3. In triglyceride experiments, the IRI response to intravenous glucose was significantly less than the IRI response to oral glucose ($p < 0.05$) even though the IR-GIP response to triglycerides was considerably greater than that to oral glucose.

DISCUSSION

In the field of gastrointestinal physiology, there remain hormonal mechanisms controlling physiologic responses that are not fully understood. One such incompletely defined mechanism involves the postulated hormone "enterogastrone," and the second relates to the release of an insulinotropic hormone from the small bowel by glucose.

Enterogastrone was a term first used by Farrell and Ivy[12] to describe a postulated hormone, believed to be released from the duodenum by fat and fat digestion products, that inhibited acid secretion from the stomach and delayed gastric emptying. Pure GIP isolated from hog duodenojejunal mucosa for its acid-inhibitory properties[13] has been demonstrated to inhibit gastrin-, histamine-, and insulin-stimulated canine gastric secretion.[11] The fact that GIP inhibited

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histamine-stimulated acid secretion was consistent with the large body of evidence that fat in the duodenum was effective in this regard.[14] GIP has also met at least two requirements of the insulinotropic hormone released from gut mucosa by glucose —namely, the insulinotropic action of the pure peptide and physiologic release of IR-GIP by glucose.

In the present study, release of IR-GIP has been shown to occur in a dose-related manner following graded oral loads of glucose or fat (figures 1 and 3).

The IR-GIP response to the doses of triglycerides used in this study was greater and more prolonged than the response to the glucose loads employed. The mean peak serum IR-GIP concentration following 100 ml. Lipomul was twice that resulting from 2.0 gm. per kilogram oral glucose (figures 1 and 3), and the time required for return to fasting levels was four and one-half to five hours, as against two to two and one-half hours following oral glucose. Because the IR-GIP secretagogues glucose and fat were administered orally, it is difficult to assign a cause for the different patterns of IR-GIP response observed. The differences in IR-GIP response may be due to direct actions of the secretagogues on GIP-releasing cells and/or may be a function of the effects of the secretagogues on the rate of gastric emptying, thus bringing fat into contact with duodeno-jejunal mucosa for extended periods of time. Studies in which IR-GIP secretagogues are infused intraduodenally are currently in progress to clarify this situation.

Data presented in figures 2B and 2C indicate that when glucose is administered to dogs by the oral route, the IRI response is magnified above the IRI response to a comparable intravenous glucose load. As illustrated in figures 2A and 2B, the serum IRI response to oral glucose is concurrent with elevation of serum IR-GIP and both reach peak values at the same time (forty-five minutes). This lends further support to the hypothesis that IR-GIP released by oral glucose augments the IRI response to the glucose stimulus.

Although exogenous porcine GIP has been shown to release insulin in man and dog, it has not been demonstrated conclusively that endogenously released GIP exerts this effect. Oral administration of fat provided a means of elevating circulating IR-GIP levels without altering serum glucose levels. Under these circumstances the IRI response to a fixed intravenous glucose load could be compared in the presence and absence of elevated IR-GIP levels. The results (figures 4B and 6) indicate that IR-GIP released by fat does exert an insulinotropic effect in the presence of an intravenous glucose load. Less augmentation of the IRI response after fat can be seen than after oral glucose (comparable serum glucose levels), even though IR-GIP levels are approximately double in the former case. It is possible that oral glucose releases insulinotropic agents in addition to IR-GIP, thus accounting for the greater augmentation of IRI release by glucose than by fat. It is also possible that insulin release is influenced by the rate of rise of circulating IR-GIP levels. If the latter is true, the results of experiments comparing oral fat (plus intravenous glucose) would be difficult to compare with those of oral glucose.

The release of IR-GIP by fat and the nature of this response are teleologically more suited to an inhibitory mechanism for acid secretion, whereas the rapid response to oral glucose is more apropos to the potentiation of a rapidly rising IRI response. It is possible that IR-GIP released by different secretagogues, although immunologically identical, are structurally different and exert different biologic actions. Chromatographic analysis of human serum following oral glucose or a mixed meal has revealed the presence of at least two molecular species which were immunologically identical to porcine GIP.[15]

Figures 4A, 5A, and 6 show that although IR-GIP levels achieved by infusion of porcine GIP were comparable to those resulting from ingestion of Lipomul, the IRI responses during GIP infusions were greater than when Lipomul was the IR-GIP secretagogue. The experimental design employed does not rule out the possibility that differences in the kinetics of elevation of serum IR-GIP were responsible for the more potent insulinotropic action of porcine GIP.

The integrated IRI response to oral glucose was not significantly different from the IRI response to intravenous GIP plus intravenous glucose (figure 6), despite the fact that circulating IR-GIP levels were considerably higher in the experiments in which GIP was infused. Species differences in potency of biologic action may be responsible for this observation, as may be the action of other insulinotropic hormones.

In conclusion, the secretagogues fat and glucose have been shown to release IR-GIP in a dose-related fashion in the dog, observations that are consistent with the gastric-inhibitory and insulin-releasing actions of porcine GIP. A comparison of the pattern of IR-GIP and IRI released in response to oral glucose is taken as strong evidence for the participation of GIP in a hormonal mechanism of insulin release operating from the small bowel. Under the experimental condi-

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tions employed, IR-GIP released by the presence of fat in the duodenum has been shown to be less potent as an insulinotropic agent than either IR-GIP released by glucose or porcine GIP.

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