Effect of Parasympathetic Denervation of Liver and Pancreas on Glucose Kinetics in Man

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The study aim was to investigate the role of the parasympathetic nervous system in the control of glucose tolerance in man. Glucose kinetics were determined during an oral glucose tolerance test (OGTT) in six subjects with truncal vagotomies and six control subjects. Basal plasma glucose levels in the two groups were equal; however, 20 to 40 minutes after the OGTT, glucose was higher in vagotomized compared with control subjects ($P < .02$). There were no differences in insulin levels between the subjects. Glucagon decreased after the OGTT in the controls, whereas in the vagotomized subjects it increased transiently and did not decrease beyond basal levels. There was no difference in basal hepatic glucose production, but suppression was greater in controls in the first 10 minutes ($P < .01$). Gut-derived glucose appearance increased faster and to a higher level (56.0 ± 8 *v* 29.7 ± 2.9 μmol/kg/min, $P < .02$) in vagotomized subjects. There were no differences in the metabolic clearance rate of glucose between the two groups. It is concluded that parasympathetic innervation of the pancreas is essential for suppression of glucagon secretion during hyperglycemia. However, abnormal glucose tolerance in vagotomized subjects is primarily due to rapid gut glucose absorption, with the denervated parasympathetic system playing only a minor role.

REFLEX ACTIVATION of the parasympathetic nervous system at the start of a meal may be important in the maintenance of normal glucose tolerance. In rats, reflex secretion of insulin in response to gustatory stimuli has been shown to be mediated by the vagus nerve.[1,2] This cephalic-phase insulin release is necessary for normal oral glucose tolerance.[3,4] Berthoud et al[3] have shown that pancreatectomized rats transplanted with islets under the kidney capsule have normal intravenous glucose tolerance but abnormal oral glucose tolerance. The transplanted islets are not innervated; hence, these rats have no cephalic insulin release. If the absent early peak of insulin is mimicked by an intravenous injection of insulin, oral glucose tolerance markedly improves but does not normalize.[3] The absence of orally mediated reflexes greatly impairs oral glucose tolerance, mainly due to a delay in the increase of peripheral glucose clearance.[5] Cephalic-phase insulin secretion has been described in humans,[6-9] but the results have been variable, possibly due to the artificial situation created by the experimental environment.[6]

Autonomic nerves can also influence glucose tolerance by modifying hepatic glucose release, with sympathetic nerves increasing and parasympathetic nerves decreasing glucose production.[10] Thus, parasympathetic denervation of both pancreas and liver may cause impaired oral glucose tolerance. It is well known that patients who have had a truncal vagotomy and pyloroplasty for the treatment of duodenal ulcer have abnormal glucose tolerance, with higher than normal glucose values at 1 hour but normal or lower than normal glucose at 2 hours.[11] This pattern may be due to more rapid glucose absorption resulting from the pyloroplasty, but a contribution from parasympathetic denervation of the liver and pancreas is possible.

The aim of this study was to investigate the role of the parasympathetic nervous system in the maintenance of normal oral glucose tolerance in man.

SUBJECTS AND METHODS

Subjects

Six subjects who had undergone a truncal vagotomy and pyloroplasty for the treatment of duodenal ulcer were studied. This procedure, if correctly performed, involves the complete parasympathetic denervation of the stomach, liver, and pancreas. Surgery was performed between 6 and 15 years before the study, and none of the subjects had a recurrence of ulcer symptoms. The subjects were overweight with a mean body mass index (BMI) of 27.6 ± 1.7 kg/m^2. They were matched for age and BMI with six control subjects who had no abdominal surgery (Table 1). Both groups contained five men and one woman. Written informed consent was obtained from each subject, and the experimental protocol was approved by the Royal Melbourne Hospital Board of Medical Research and Human Research Ethics Committee.

Oral Glucose Tolerance Test

Subjects fasted overnight, and on the morning of the test reported to the Burnet Clinical Research Unit at the Royal Melbourne Hospital where the studies were performed. Under local anesthesia, a short intravenous catheter (Jelco; Johnson & Johnson, Chiodya-Yu, Japan) was inserted into a cubital vein and used for infusion of glucose tracer. A 21-gauge scalp-vein needle was inserted into a vein in the dorsum of the contralateral hand for sampling of arterialized venous blood. This line was kept patent by a slow infusion of normal saline. A blood sample was taken, and a primed continuous infusion of 6,6-dideuterated glucose ([6,6-D_2]-glucose; Cambridge Isotope Laboratories, Cambridge, MA) was commenced, driven by a Gilson Minipuls 2 pump (Gilson, Villiers-le-bel, France). All subjects were administered a bolus of 0.67 g [6,6-D_2]-glucose followed by a constant infusion of 7.2 ± 0.7 mg/min for the duration of the experiment. Ninety minutes were allowed for equilibration of the tracer. Four blood samples were then taken at 10-minute intervals for measurement of glucose and the percent enrichment of [6,6-D_2]-glucose. At 120 minutes, the subjects drank 75 g orange-flavored glucose to which 100 μCi [6-^{3}H]-glucose (NEN-Dupont Research Products, Boston, MA) had been added. Blood samples were taken at 10-minute intervals for the next 2 hours for determination of glucose, percent enrich-

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Submitted October 9, 1995; accepted February 28, 1996.

Supported by a program grant from the National Health and Medical Research Council of Australia.

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0026-0495/96/4508-0013$03.00/0

Table 1. Clinical Characteristics of the Subjects

Characteristic	Vagotomized (n = 6)	Control (n = 6)
Age (yr)	57.0 ± 3.0	51.0 ± 5.0
Weight (kg)	77.6 ± 5.3	88.5 ± 2.4
BMI (kg/m^2)	27.6 ± 1.7	29.6 ± 1.5
Fasting plasma glucose (mmol/L)	5.4 ± 0.4	5.1 ± 0.2
Fasting plasma insulin (mU/L)	7.8 ± 1.5	7.9 ± 1.4

ment of [6,6-D_2]-glucose, and specific activity of [6-^{3}H]-glucose. Samples were also taken at 2, 4, and 6 minutes following the drink in five vagotomized subjects and four controls, and then insulin samples were taken at 30-minute intervals for the rest of the study in all subjects. Samples for glucagon estimation were collected in 10% vol/vol trasylol (Aprotinin; Bayer, Leverkusen, Germany) at 30-minute intervals starting at 90 minutes. At the end of the experiment, the infusion rate of [6,6-D_2]-glucose was accurately determined. Aliquots of the ingested glucose were taken from each experiment for measurement of specific activity of the drink.

Analytical Procedures

Glucose was assayed with a Yellow Springs glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH). Plasma insulin level was measured by immunoassay using a second antibody to separate bound and free insulin (Pharmacia, Uppsala, Sweden). The interassay coefficient of variation for this assay is 16.7% at 10 μU/mL and 7.6% at 112 μU/mL. Plasma glucagon level was measured using a radioimmunoassay with dextran-charcoal separation of bound and free fractions (interassay coefficient of variation, 13.8% at 137 pg/mL). To determine the percent enrichment of [6,6-D_2]-glucose and the specific activity of 6-^{3}H-glucose, 500 μL plasma was deproteinized with 500 μL 0.3-mol/L $Ba(OH)_2$ and 500 μL 0.3-mol/L $ZnSO_4$. The supernatant was passed down an ion-exchange resin (Ag-2X8; Bio-Rad, Richmond, CA) to remove charged labeled metabolites of glucose. The columns were washed with 4 mL distilled water, and the eluant was collected in scintillation vials that were then dried in an oven to remove tritiated water. Distilled water (1.0 mL) was then added to redissolve the glucose, and the samples were divided into two aliquots. One hundred microliters was placed in limited-volume inserts (Alltech Association, Homebush, Australia) and dehydrated before being converted to the pentaacetate derivative using pyridine and acetic anhydride. The derivatized glucose was assayed with a GCMS-QP2000 (Shimadzu, Kyoto, Japan) using the selected ion monitoring mode to determine the relative abundance of the selected ions, m/z 98 and m/z 100. Distilled water (3.1 mL) was added to the remaining 900 μL reconstituted eluant together with 10 mL scintillant (Ready Value; Beckman Instruments, Fullerton, CA) and counted in the gel phase using a beta counter.

Calculations

Glucose kinetics basally and during the oral glucose tolerance test (OGTT) were calculated from equations derived by Steele et al.[12] Briefly, the total rates of glucose appearance and disappearance were determined from the change in percent enrichment of [6,6-D_2]-glucose, using a modified one-pool model with 0.65 as the rapidly mixing portion of the glucose pool and estimating the glucose space as 25% of body weight (the extracellular space). The metabolic clearance rate of glucose was calculated as the rate of disappearance divided by the prevailing glucose concentration. The plasma rate of appearance of the ingested [6-^{3}H]-glucose was determined by transposition of the Steele equation.[12] The rate of appearance of the ingested glucose was determined from the known specific activity of the drink. Hepatic glucose production was calculated as the total rate of appearance of glucose minus the rate of appearance of gut-derived glucose. This approach has been validated in dogs[13] and rats.[14]

Statistical Analysis

Statistical significance was determined using the Mann-Whitney test.

RESULTS

Plasma glucose, insulin, and glucagon levels are shown in Fig 1. There was no difference in basal glucose levels between vagotomized and control subjects; however, during the OGTT, glucose levels were significantly higher in the vagotomized group between 20 and 40 minutes after the glucose load ($P < .02$). Glucose peaked 20 minutes earlier in vagotomized subjects versus controls. There were no differences in insulin levels between the subjects either basally or during the OGTT, although there was a trend for insulin levels to mirror the changes in glucose concentrations (Fig 1). No cephalic insulin release could be detected in either the control or vagotomized subjects. Insulin levels in controls were 11 ± 1 mU/L before the OGTT, 11 ± 1 2 minutes after the OGTT, and 12 ± 2 at 4 minutes. In vagotomized subjects, basal insulin values were 9 ± 2 before, 9 ± 2 at 2 minutes and 13 ± 4 at 4 minutes. Basal glucagon levels were not different in the two groups, but after the OGTT glucagon decreased by 60% in control subjects, whereas in vagotomized subjects it increased dramatically, reaching a peak of 78 ± 8 pg/mL as compared with 40 ± 8 pg/mL in controls ($P < .02$). Glucagon levels in vagotomized subjects remained higher than in controls throughout the rest of the study and did not decrease beyond basal levels (Fig 1).

There was a tendency for vagotomized subjects to have higher basal hepatic glucose production than control subjects (10.3 ± 1.2 *v* 8.6 ± 0.5 μmol/kg/min, respectively; however, this was not statistically significant ($P = .3$; Fig 2). Suppression of hepatic glucose production was greater in controls in the first 10 minutes ($P < .01$), but there was no difference in suppression between controls and vagotomized subjects for the rest of the test.

The rate of gut-derived glucose absorption (Ra gut) increased faster and to a greater extent in vagotomized versus control subjects (Fig 3). The vagotomized group reached a peak of 56.0 ± 8.5 μmol/kg/min 30 minutes after glucose ingestion, as compared with the control group, which reached a peak value of 29.7 ± 2.9 μmol/kg/min ($P < .02$) 30 minutes later. The vagotomized group had a significantly higher Ra gut between 20 and 40 minutes after the glucose load compared with the controls. Ra gut then declined more rapidly in vagotomized subjects, to a value of 5.3 ± 3.0 μmol/kg/min at 240 minutes, compared with 21.7 ± 1.3 μmol/kg/min in control subjects ($P < .01$).

The rate of glucose disappearance and the metabolic clearance rate of glucose for the two groups are illustrated in Fig 4. There was a dramatic increase in the rate of glucose disappearance in vagotomized subjects 40 minutes

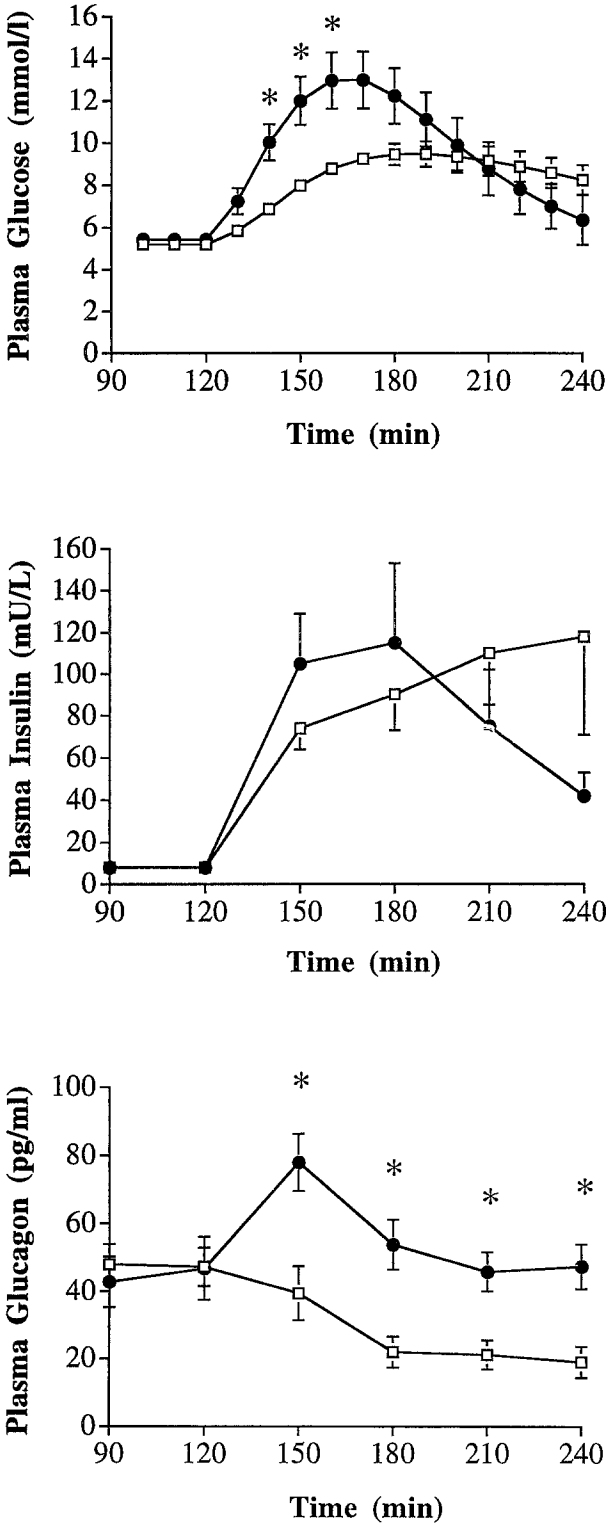

Fig 1. Plasma glucose, insulin, and glucagon concentrations under basal conditions and during the OGTT in (●) vagotomized and (□) control subjects. *$P < .05$.

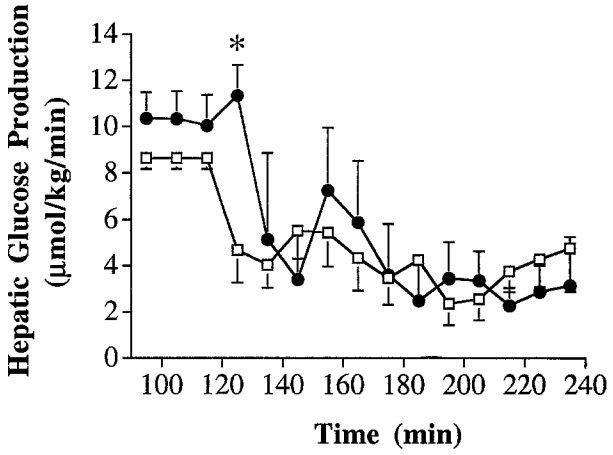

Fig 2. Rates of hepatic glucose production basally and during the OGTT in (●) vagotomized and (□) control subjects. Rate measurements over a 10-minute period (eg, between 120 and 130 minutes) are plotted in the middle of that period (ie, at 125 minutes). *$P < .05$.

after the start of the OGTT, which tended to remain higher than that in the control subjects for the next 50 minutes. There were no differences in the metabolic clearance rate between the two groups either basally or during the study.

DISCUSSION

Studies in rats suggest that the autonomic nervous system is important for the maintenance of normal oral glucose tolerance.[5] Whether similar reflexes operate in man is not known. The aim of the present study was to investigate the role of the autonomic nervous system in glucose homeostasis using individuals who have had parasympathetic denervation of the pancreas and liver as a result of therapy for a duodenal ulcer. These subjects are known to have an abnormal glucose tolerance curve. The results clearly show that this abnormal glucose tolerance is largely due to a more rapid appearance of ingested glucose in vagotomized

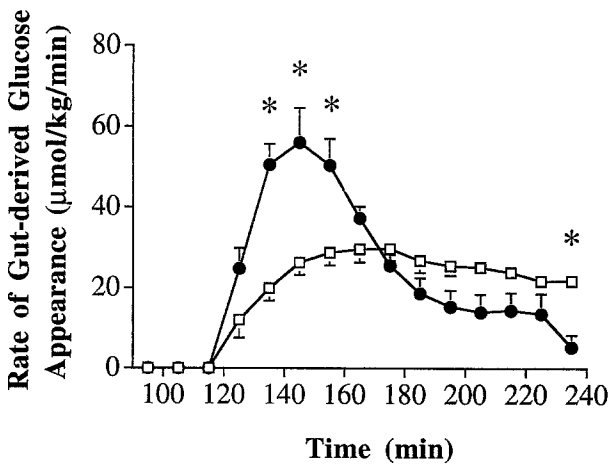

Fig 3. Ra gut basally and during the OGTT in (●) vagotomized and (□) control subjects. Rate measurements over a 10-minute period (eg, between 120 and 130 minutes) are plotted in the middle of that period (ie, at 125 minutes). *$P < .05$.

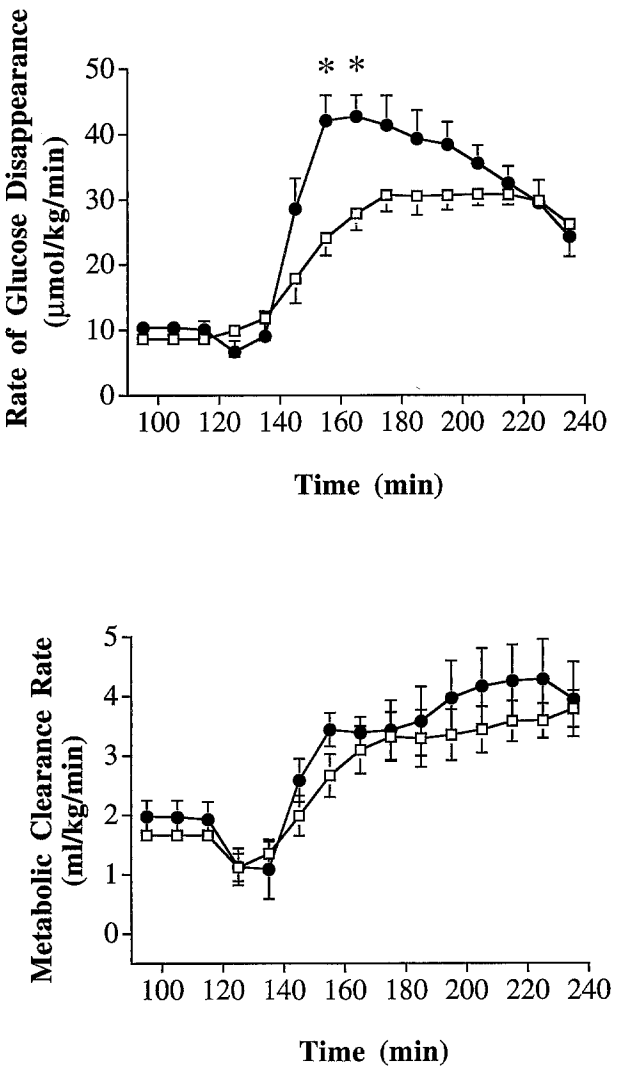

Fig 4. Rate of glucose disappearance and the metabolic clearance rate of glucose under basal conditions and during the OGTT in (●) vagotomized and (□) control subjects. Rate measurements over a 10-minute period (eg, between 120 and 130 minutes) are plotted in the middle of that period (ie, at 125 minutes). *$P < .05$.

subjects. This is probably caused by the pyloroplasty that is performed with the truncal vagotomy to allow gastric drainage following parasympathetic denervation of the stomach. We attempted to detect cephalic-phase insulin release (CPIR) in our subjects; however, the results were inconclusive. CPIR has been shown to occur in humans,[8,15,16] although it is more difficult to detect than in rats.[6,16] Variability, both between and within studies, and the low amplitude of the responses make CPIR studies difficult to interpret.[8] It is possible that a lack of food aromas and other meal-related stimuli, coupled with the clinical setting, prevented CPIR in the control group in this study. It is also possible that extraction of insulin by the liver may have masked CPIR. It has been suggested that stress caused by the experimental situation may also mask this reflex in man.[6] These effects may have minimized differences in CPIR and carbohydrate metabolism in the two groups.

In contrast to the lack of effect of vagotomy on insulin secretion, a dramatic difference in glucagon release was demonstrated. Control subjects showed the expected decrease in glucagon following ingestion of glucose. Following glucose ingestion in vagotomized subjects, there was a marked increase in glucagon, and despite higher glucose levels, glucagon did not decrease beyond basal. This grossly abnormal regulation of glucagon secretion suggests that the vagus nerve is involved in the suppression of glucagon release by glucose. It is known that activation of the sympathetic nervous system powerfully stimulates glucagon secretion.[17] However, the role of the vagus nerve is controversial. There is evidence that during hypoglycemia, vagal stimulation is necessary for glucagon secretion.[18] In contrast, suppressing parasympathetic activity with atropine delays suppression of glucagon secretion following oral glucose,[19] suggesting that in the presence of hyperglycemia, vagal stimulation promotes suppression of glucagon secretion. However, in this study it was possible that administration of atropine may have delayed gastric emptying, resulting in a slower rate of glucose absorption.[19] In the present study, gastric emptying was more rapid and thus cannot be the explanation for the failure of glucagon suppression. Although it is likely that parasympathetic denervation of the pancreas was responsible for the elevated glucagon levels seen in the vagotomized subjects, we cannot exclude the possibility that denervation of other organs such as the stomach was partly responsible.

These results raise the intriguing possibility that parasympathetic stimulation of the α cell has opposite effects on glucagon secretion depending on the level of plasma glucose. Thus, during hypoglycemia vagal stimulation is important for glucagon secretion,[18] whereas, as shown in the present study, during hyperglycemia vagal stimulation is essential for suppression of glucagon secretion.

Lautt[20] proposed that parasympathetic nerve activation is essential for the insulin-stimulated increase in hepatic glucose uptake. Using dogs, Chap et al[21] demonstrated a decrease in hepatic glucose uptake following treatment with atropine. In the present study, the measured Ra gut is the difference between true gut glucose absorption and first-pass hepatic glucose uptake. The increase in Ra gut could therefore be partly due to reduced hepatic glucose uptake. There is no way to distinguish these two possibilities from the present study. The fact that Ra gut was lower in the second half of the study suggests that the total amount of glucose that appeared in the circulation during the study was the same in vagotomized and control subjects, consistent with rapid gut glucose absorption rather than reduced hepatic glucose uptake.

There was a trend for basal hepatic glucose production to be higher in vagotomized subjects and for suppression of hepatic glucose production to be delayed by 10 minutes, suggesting that a lack of parasympathetic innervation of the liver has a small effect on hepatic glucose production. However, hepatic glucose production was suppressed equally

in controls and vagotomized subjects, and toward the end of the study the rates were not different despite the fact that in vagotomized subjects glucagon levels were higher while insulin levels were lower, resulting in different insulin to glucagon ratios (7.9 ± 2.2 *v* 0.9 ± 0.2, $P < .02$). This could be due to the residual effects of the suppressive action of hyperglycemia on hepatic glucose production[22] in vagotomized subjects. The metabolic clearance rate of glucose was not different in the two groups, suggesting that in man there is little effect of the autonomic nerves on peripheral glucose disposal. The higher rate of disappearance in vagotomized subjects was entirely due to the hyperglycemia forcing more glucose into tissues by a mass-action effect. Later in the study, plasma glucose returned to normal in the vagotomized group, and this is likely responsible for the equivalent rate of glucose disappearance in the control and vagotomized subjects.

It is concluded that parasympathetic nerve innervation of the pancreas is likely essential for suppression of glucagon secretion during hyperglycemia. However, abnormal glucose tolerance in subjects who have had a truncal vagotomy is primarily due to rapid glucose absorption across the small intestine, with the denervated parasympathetic nervous system playing only a minor role in influencing blood glucose concentration.

ACKNOWLEDGMENT

We gratefully acknowledge the excellent technical assistance of Lisa Wilson.

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