The role of cholinergic mechanisms in the control of gallbladder emptying has not been defined. In this report a scintigraphic technique using 2,6-dimethylphenylcarbamoylmethyl iminodiacetic acid labeled with technetium 99m to visualize the gallbladder and its emptying and diethylenetriamine pentaacetic acid labeled with indium 111 to visualize the stomach and its emptying was used to study 43 normal subjects and 18 patients with vagotomies. Direct cholinergic stimulation with bethanechol induced significant gallbladder emptying. Cholinergic blockade with atropine sulfate decreased the gallbladder emptying responses to a liquid meal, a solid meal, and the infusion of cholecystokinin-octapeptide. In patients with vagotomies, gallbladder emptying was decreased despite accelerated gastric emptying of an ingested liquid meal. The gallbladder emptying response to cholecystokinin-octapeptide was augmented in patients who had undergone vagotomy. These studies suggest that cholinergic receptors may be important in the regulation of human gallbladder emptying.

The effects of vagotomy on gallbladder motor function have not been elucidated clearly. Although some observers have reported gallbladder dilatation and decreased gallbladder contractions after vagotomy, presumably due to cholinergic disruption (1–8), others have reported no changes (9–12). There are several potential explanations for this discrepancy. First, experiments have been conducted in different animal species. Second, there have been differences in the surgical procedures under investigation. Truncal, selective, and highly selective vagotomies have been intermingled with gastrectomy and pyloroplasty. And third, the methods used to measure gallbladder emptying have been indirect, using either roentgenographic techniques to estimate gallbladder area and volume (13,14) or duodenal aspiration to quantitate duodenal outputs of substances such as bilirubin, bile salts, and indocyanine green (15–17).

Although direct quantitative tests for gastric emptying have been in use for years, measurements of gallbladder emptying have not kept pace. Recently, however, γ-emitting, hepatocystic radionuclides, which are taken up by the liver and concentrated in the gallbladder, have been used to measure gallbladder emptying directly (18–23). By using a second γ-emitting radionuclide to label a test meal, gastric emptying can be quantitated at the same time (24). Therefore, cholecystogastric scintigraphy provides a tool whereby gallbladder evacuation and gastric emptying of a test meal can be studied simultaneously, noninvasively, and quantitatively in normal subjects and in patients who have undergone vagotomies.

The purpose of this study was to determine whether gallbladder emptying in humans is affected by cholinergic stimulation, cholinergic blockade, or vagotomy.

Materials and Methods

This project was approved by the Human Research Review Committee of Temple University School of Medicine in July 1981. Written, informed consent was obtained from all subjects before they were studied.

A tubeless scintigraphic technique was used to measure gallbladder emptying in 43 normal male subjects (age range 23–39 yr, mean age 27.2 yr) and 18 patients who had undergone truncal vagotomies (11 men and 7 women, age range 34–59 yr, mean age 45.3 yr). Normal volunteers had

Abbreviations used in this paper: DTPA, diethylenetriamine pentaacetic acid; HIDA, 2,6-dimethylphenylcarbamoylmethyl iminodiacetic acid.
no history of gastrointestinal symptoms or surgery. In 13 of
the vagotomy patients, vagotomies were accompanied by a
left gastrectomy and Billroth II gastroduodenostomy. In 5
patients, a pyloroplasty had been performed. The gall-
bladder scanning agent, 2,6-dimethylphenylcarbamoyl-
methyl iminodiacetic acid labeled with technetium 99m
($^{99m}$Tc-HIDA), was used to identify the gallbladder and
quantitate its evacuation in response to cholinergic stim-
ulation by bethanechol, ingestion of liquid and solid test
meals, and intravenous infusion of cholecystokinin-
octapeptide (Squibb Inc., Princeton, N.J.). The effects of
cholinergic blockade with atropine sulfate were tested also.
$^{99m}$Tc-HIDA was prepared from a commercially avail-
able kit (Medi-Physics, Emeryville, Calif.).

Studies were performed in subjects who had fasted
overnight. They were positioned supine under the detector
of a $\gamma$-camera, which was fitted with a diverging collimator
on line to a scintigraphic data analyzer (Hewlett-Packard
5407A, Waltham, Mass.). Five milli-Curies of $^{99m}$Tc-HIDA
(5 mg) was administered intravenously and count rates
were recorded continuously for 60 min with the
photopeak window set at 140 keV $\pm$ 14%. After 60 min,
when the $^{99m}$Tc-HIDA activity was maximal in the gal-
bladder, as confirmed by time-activity counts, gallbladder
emptying responses to a multicomponent liquid meal or
an equivalent solid meal, cholinergic stimulation
with bethanechol, or intravenous infusion of cholecystokinin-
peptide were tested. In some subjects, gastric emptying
of the liquid test meal was measured simultaneously
by mixing the liquid test meal with 250 &mu;Ci of
diethylenetriamine pentaacetic acid labeled with indium
$^{111}$ ($^{111}$In-DTPA). The stomach was identified by imaging
the $^{111}$In-DTPA activity in the indium window set at 171
keV $\pm$ 6%. $^{99m}$Tc-HIDA, $^{111}$In-DTPA, or both agents
could be imaged by using the two windows on the $\gamma$-camera.

Using a light pen, areas of interest corresponding to the
gallbladder and stomach were outlined. Corrections were
made for background, radioactive decay, and backscatter.

In some studies, cholinergic blockade was tested by ad-
ministering the antimuscarinic agent atropine sulfate in-
travenously at a dose of 12 &mu;g/kg body wt $\cdot$ min. Infusions
of atropine began 55 min after the administration of
$^{99m}$Tc-HIDA, when $^{99m}$Tc activity was maximal in the gal-
bladder, but 15 min before the test meal or other
stimuli were administered.

Immediately after ingestion of the test meal or adminis-
tration of bethanechol (5 mg, subcutaneously) or chole-
cystokinin-octapeptide (5 &mu;g/kg body wt $\cdot$ min), and at
15-min intervals for the next 2 h, $^{99m}$Tc or $^{111}$In counts, or
both, were recorded for 1-min time periods in supine
subjects who were instructed to assume the upright posi-
tion between counting periods. Cumulative gallbladder
emptying over a specific time period was obtained by
dividing the decrease in $^{99m}$Tc activity over the gallbladder
area of interest by the initial maximal $^{99m}$Tc activity and
then multiplying by 100 to obtain the percentage of gall-
bladder emptying. Cumulative gastric emptying of the test
meal was obtained by dividing the decrement in $^{111}$In activity
over the gastric area of interest by the initial maximal $^{111}$In activity and then multiplying by 100.

The multicomponent liquid test meal consisted of 300
ml of Meritene (Doyle Pharmaceutical Co., Minneapolis,
Minn.) and 15 ml of Lipomul (The Upjohn Company,
Kalamazoo, Mich.). The chemical composition of the test
meal was 36 g of carbohydrate, 10 g of protein, and 20 g of
fat. For measurement of gastric emptying, this meal was
mixed with 250 &mu;Ci of $^{111}$In-DTPA. An equivalent solid
meal consisted of 50 g of egg white, 20 g of chicken liver,
35 g of white toast, 14 g of sugar, 10 g of margarine, 100 g
of half and half, and 3 g of cocoa. The chemical composi-
tion of the solid test meal was 40 g of carbohydrate, 15 g of
protein, and 20 g of fat.

Doses of bethanechol, cholecystokinin-octapeptide, and
atropine sulfate to be used in this study were selected
based on previous studies in our laboratory. When
bethanechol was administered subcutaneously at a dose of
10 mg, the subjects complained of abdominal cramps and
urinary frequency. Doses of cholecystokinin-octapeptide
at 0.5, 1.0, and 5.0 &mu;g/kg body wt $\cdot$ min all produced
similar gallbladder emptying curves. Therefore, this maxi-
mal dose was chosen. We have used atropine sulfate at a
dose of 12 &mu;g/kg body wt $\cdot$ min previously in order to block
the acid secretory response to sham feeding and to dimin-
ish resting lower esophageal sphincter pressures and
responses to cholinergic agonists. In this study atropine
effects were confirmed by an increased heart rate and a
change in pupil size.

Evaluations of scintigraphic data were performed by
L. S. M. who had no knowledge of which test meal or
pharmacologic agent had been administered. Statistical
analyses of gallbladder and gastric emptying curves were
performed by the Student's t-test (25). Cholecystogastric
scintigraphy resulted in radiation exposures of 103 mrads
to the whole body and 2300 mrads to the lower large
intestine, the target organ, per study. Cholecystoscintigraphy,
performed alone, resulted in lower exposures. This is
similar to a radiation exposure of $\sim$5 rads/min for fluoro-
cscopic studies (26).

Results

In normal subjects, the gallbladder emptied
rapidly after ingestion of a standard multicomponent
liquid meal. Serial scintigrams obtained from a sin-
gle normal subject immediately, 60 min, and 120
min after administration of the test meal are shown
(Figure 1). In this subject 74% of the gallbladder
actively had cleared by 60 min and 80% by 120 min.
Significant gallbladder emptying was seen, not only
after ingestion of a standard multicomponent liquid
test meal, but also after the administration of
bethanechol in studies performed on 10 normal
subjects (Figure 2). No gallbladder emptying was
observed after a subcutaneous injection of normal
saline. The maximal gallbladder emptying responses
to the liquid test meal and bethanechol were 77.9% 
$\pm$ 3.0% and 39.2% $\pm$ 8.3%, respectively.

Cholinergic blockade with atropine sulfate de-
creased the gallbladder emptying response to a stan-
dard multicomponent liquid meal in 10 normal
volunteers (Figure 3). Emptying of the liquid meal from the stomach was also diminished significantly by the administration of atropine (Figure 3). Atropine decreased the maximal gallbladder emptying at 60 min from 71.3% ± 3.0% to 24.6% ± 2.6% (p < 0.01), and at 120 min from 79.9% ± 3.8% to 60.1% ± 3.2% (p < 0.05). Corresponding maximal cumulative gastric emptying rates were 44.6% ± 2.2% and 27.9% ± 1.8% (p < 0.05) at 60 min, and 80.1% ± 2.0% and 52.7% ± 2.7% (p < 0.05) at 120 min. The gallbladder emptying response to an equivalent multicomponent solid test meal in 10 normal subjects also decreased (Figure 4). Atropine decreased the maximal cumulative gallbladder emptying rate at 120 min from 74.6% ± 6.8% to 39.0% ± 8.8% (p < 0.05).

In 18 patients who had undergone truncal vagotomy to treat peptic ulcer disease, gallbladder emptying in response to a multicomponent standard liquid test meal was decreased significantly compared with normal control subjects (Figure 5). The cumulative maximal gallbladder emptying rate at 120 min was 60.4% ± 4.5% compared with 79.9% ± 3.8% (p < 0.05) in controls. In contrast, gastric emptying of the liquid test meal was accelerated, especially during the first 60 min (Figure 5). At 60 min after ingestion of the test meal, the cumulative gastric emptying rate was 44.9% ± 8.5% in control subjects and 76.2% ± 6.4% (p < 0.01) in patients who had undergone vagotomy. No significant differences were apparent between the patients with partial gastrectomy and Bilroth II gastroenterostomies and those with pyloroplasties.

In 10 normal subjects, intravenous infusion of cholecystokinin-octapeptide stimulated significant gallbladder emptying (Figure 6). The gallbladder emptying response to cholecystokinin was decreased significantly by cholinergic blockade with atropine. Cumulative gallbladder emptying was decreased from 59.8% ± 6.0% to 22.8% ± 8.2% (p < 0.05) at 15 min, and from 82.4% ± 9.0% to 66.0% ± 9.2% (p < 0.05) at 60 min. A higher dose of cholecystokinin-octapeptide was not tested.
trast, the gallbladder emptying response to cholecystokinin-octapeptide was augmented significantly in 10 patients who had undergone truncal vagotomy (Figure 6). Whether these patients had undergone Bilroth II gastrojejunostomies or pyloroplasties made no difference in the responses.

**Discussion**

The results of this study demonstrate the presence of cholinergic receptors that may affect gallbladder emptying. Direct cholinergic stimulation by bethanechol produced significant gallbladder emptying. Cholinergic blockade with atropine sulfate diminished the gallbladder emptying responses to both liquid and solid meals. Atropine also delayed the gastric emptying of a liquid test meal. In patients with vagotomies, gallbladder emptying was delayed significantly despite accelerated gastric emptying. Gallbladder emptying was stimulated by the intravenous infusion of cholecystokinin-octapeptide. In normal subjects, this effect was decreased significantly by the administration of atropine. In contrast, the gallbladder emptying response to cholecystokinin-octapeptide was augmented in patients with vagotomies.

Stimulation of cholinergic receptors by bethanechol and meal ingestion and, possibly, even by cholecystokinin-octapeptide may be an important factor in the regulation of gallbladder emptying in humans. Interestingly, however, cholinergic stimulation does not seem to account for the entire gallbladder emptying response, as gallbladder emptying was significantly greater after meals and administration of cholecystokinin-octapeptide than after stim-
ulation by bethanechol alone. Further evidence for the importance of cholinergic receptors is provided by the observations that gallbladder emptying was decreased during administration of atropine and after vagotomy.

It has been widely accepted that gallbladder emptying is controlled by the release of cholecystokinin from the small bowel mucosa. How then can we explain our observations or integrate them into the overall scheme of the regulation of gallbladder emptying? Atropine sulfate is a cholinergic antagonist. It may act solely by blocking cholinergic receptors. However, one cannot exclude several other possibilities. Atropine may diminish the release of endogenous cholecystokinin or some other important peptide from the small intestinal mucosa or it may act by unmasking some pathways that are inhibitory for the gallbladder emptying response to meals or cholecystokinin.

Vagotomy, also, may have multiple effects on gallbladder emptying. First, vagal stimulatory pathways might be disrupted by vagotomy. However, the presence of vagal inhibitory pathways, which might also be blocked, cannot be excluded. This might explain the augmented gallbladder emptying response to exogenous cholecystokinin that was observed in patients who had undergone vagotomy. Second, performing a vagotomy might diminish the release of endogenous cholecystokinin or other peptides from the small intestinal mucosa. Third, the surgical procedure Billroth II gastrojejunostomy, which accompanied vagotomy in many of the study patients, bypasses a significant length of the peptide-rich proximal small intestinal mucosa. This might result in a decreased release of cholecystokinin or other peptides. Against this third explanation is the observation that there was no difference between

Figure 5. Composite results of gallbladder and gastric emptying studies in 18 vagotomy patients (solid symbols) and 10 normal control subjects (open symbols). Left panel. Cumulative gallbladder emptying in percent is shown on the vertical axis and time in minutes after meal ingestion is shown on the horizontal axis. Each point represents the mean ± SEM. Right panel. Cumulative gastric emptying in percent is shown on the vertical axis and time in minutes after meal ingestion on the horizontal axis. Each point represents the mean ± SEM.

Figure 6. Composite results of gallbladder emptying responses to intravenous infusion of cholecystokinin-octapeptide in 10 normal subjects. Control studies are designated by open circles and studies on a separate day during administration of atropine by solid circles. Studies performed in 10 patients with truncal vagotomies are designated by open squares. Cumulative gallbladder emptying in percent is shown on the vertical axis and time in minutes after beginning the infusion of cholecystokinin (CCK) is shown on the horizontal axis. Each point represents the mean ± SEM.
patients with vagotomy and pyloroplasty versus patients with vagotomy and Bilroth II gastrojejunostomy in terms of the gallbladder emptying responses to meals or cholecystokinin-octapeptide.

One might speculate that the observed effects on gallbladder emptying were due to changes in the rate of stomach emptying. This would not fit with the data. Although both gallbladder and gastric emptying were slowed by the administration of atropine, gallbladder emptying decreased despite accelerated gastric emptying in patients with vagotomy. It is unlikely that the ages of the normal subjects and the vagotomy patients are responsible for the observed differences in gallbladder emptying. In a previous study, we reported that age did not seem to affect gallbladder emptying (24).

The answers to many of these questions about the control of gallbladder emptying await the availability of a reliable radioimmunoassay for cholecystokinin. The observations reported herein were made possible by recent advances in our ability to measure gallbladder emptying. Cholescintigraphy has been validated by several investigators as a useful method to evaluate gallbladder emptying (18-24).

Scintigraphic techniques have several advantages over other methods that have been used to test gallbladder emptying. Roentgenographic (13,14) or ultrasonographic (27) techniques estimate gallbladder volumes from serial planimetric measurements of radiopaque or sonolucent areas, using formulas that depend upon assumed, theoretical geometric models. Unfortunately, the shape of the gallbladder may change with contraction. These methods equate changes in gallbladder area or volume with emptying, an assumption that has not been tested. The radiation burdens associated with roentgenographic methods are significantly higher than those associated with scintigraphy (26). Also, roentgenographic and ultrasonic techniques do not provide a simultaneous measure of gastric emptying (24).

Marker infusion or aspiration systems (15-17), which utilize duodenal output of bile as an index of gallbladder emptying, require oroduodenal intubation and intravenous infusion of large volumes of markers, which in themselves might alter normal biliary tract physiology. Aspiration methods assume a steady state and cannot differentiate reliably between bile emptied from the liver and the gallbladder. Duodenal outputs are expressed relative to intravenous infusion rates. Thus, the estimates of gallbladder emptying are indirect and dependent upon efficient duodenal collection. Again, gastric emptying is not measured.

In summary, these studies suggest that cholinergic mechanisms may be important in the regulation of gallbladder emptying in humans. The specific details of the system remain to be clarified.

References