原　　　　著

Effects of Duodenal pH Levels on Secretin-secretion in the Fasting Phase of Dogs

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Received for Publication, Sept. 5, 1983.

Intraluminal pH of the duodenum strongly influences gut hormons-secretion, especially secretin. It is essential and informative to measure duodenal pH simultaneously when the blood is aspirated for secretin determination. Evaluations of duodenal pH have been done either by aspirating the duodenal contents[2,3] or by measuring of pH in situ using a glass membrane electrode[10,11].

We developed a new method for measuring duodenal bulbar pH using an ion-sensitive field effect transistor sensor.

This method was applied for the determination of the diurnal changes in duodenal bulbar pH; the effects of these changes on secretin release in the fasting phase were evaluated in conscious dogs.

Materials and Methods

Animal preparations

Duodenal cannulation

Fifteen healthy mongrel dogs of both sexes weighing 12 to 18 kg body weight were used. Under laparotomy, a duodenal stainless steel cannula (6 mm in diameter) connected with a polyvinyl chloride tube at the distal end was inserted through the second portion of the duodenum and guided into the duodenal bulb (Fig. 1). It was confirmed during laparotomy that the tip of the polyvinyl chloride tube located within 4 cm distal to the pylorus ring. The distal end of the cannula was exteriorized through a right flank stub wound and sewn permanently. A recovery period of at least two weeks was allowed before these dogs were used for experiment.

Venous cannulation

Three dogs among them were used for the assessment of fasting secretin levels. A 8 Fr sized silastic tube was placed into the superior caval vein through the external jugular vein for
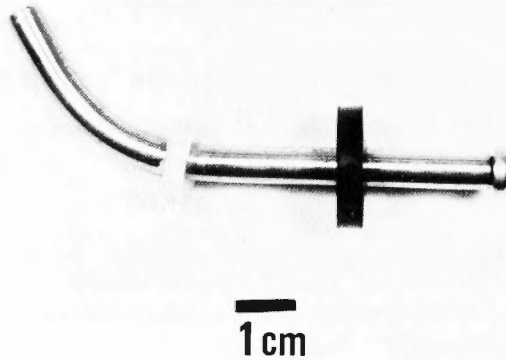
1 cm

Fig. 1. A stainless steel duodenal cannula connected to a silastic tube. It was inserted
through the second portion of the duodenum and guided into the bulb. A
purse-string suture was inserted into the duodenal wall around the cannula and
ligated. The cannula was passed through omental sheath and was exteriorized
through a right flank stub wound and permanently fixed. The sensor was in-
serted through this cannula.

blood sampling. The distal end of this tube was exteriorized through a stub wound on the back
of the dogs. This tube was filled with saline containing heparin until survey, and was protected
from scratching or chewing by a special jacket.

Measurement of pH

The ion-sensitive field effect transistor sensor was developed and manufactured by Kuraray.
Co., LTD., Osaka, Japan. The flexible sensor (30 cm in length and 1.5 mm in diameter) was
equipped at its distal end with an ion effect transistor (0.5 mm × 1.5 mm) (Fig. 2). The sensor was
connected to a pH meter (KR-500 pH/pCO_2 monitor. Kurarey Co., LTD., Osaka, Japan).
The output of the pH meter was recorded by a pen recorder (KR-102 recorder. Kurarey Co.,
LTD., Osaka, Japan). The sensor was standardized at 37°C using commercial buffer solutions
of pH4.01 and pH6.86 (Kanto Chemical Co., LTD., Tokyo, Japan) before each test. To evaluate
the response time of the sensor, the pH meter was calibrated using these buffer solutions. The
pH values were recorded at a speed of 4 cm per minute. The response time obtained was shorter
than 0.5 second for a shift between these pH values (Fig. 3). The sensor was stable enough to
withstand strong impact of its tip against the test tube wall without affecting the pH values.

The sensor was inserted into the duodenal bulb through a duodenal cannula and was fixed
so that the sensor tip should be exposed more than 2 cm long from the cannula tip. Namely, the
sensor tip was placed within 2 cm distal to the pylorus ring. After fasting for more than 16 hours,
dogs were given a liquid meal composed of 40 g of minced meat (Vita-one, Nippon Pet Food, Co.,
LTD., Tokyo, Japan) per kg. body weight mixed with 20ml of water per kg. body weight. The
pH of the food was adjusted to pH6.30. The pH was recorded at a speed of 8 cm per hour while
measuring the diurnal changes. The distortion of the pH values over 24 hours remained within
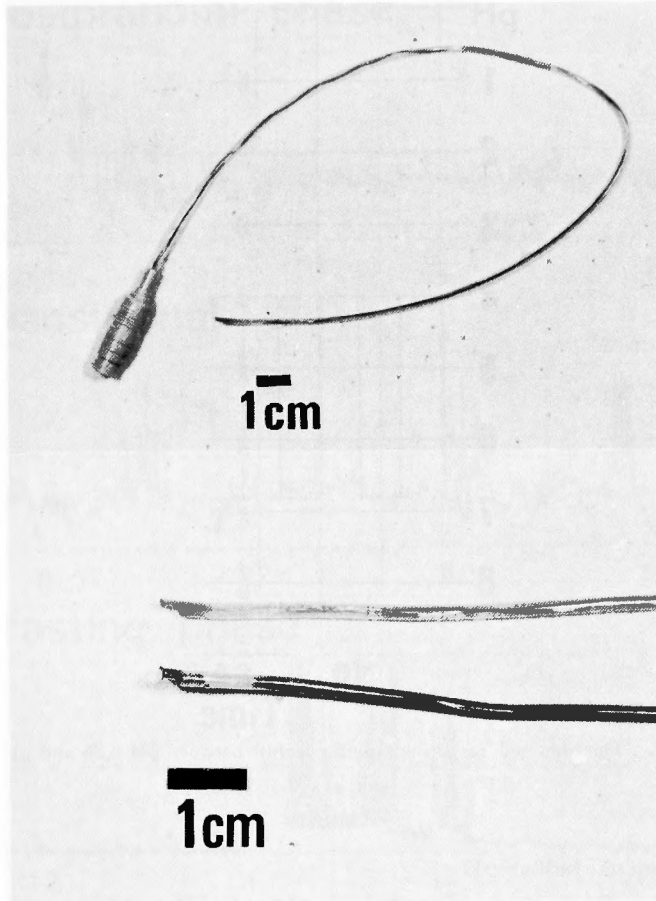0.2 pH units.

Fig. 2. An ion sensitive field effect transistor sensor used for measurement of pH. A transistor is attached at the end.

Radioimmunoassay of secretin

Blood(1 ml) was aspirated into test tubes containing 0.02 ml of heparin and trasylol 250 U and then the tubes were centrifuged at 3,000 rpm for 15 minutes. Serum was stored at -20°C until assay. Immunoreactive secretin was measured using a Secretin Radioimmunoassay Kit (Daiichi Radioisotope Laboratory Co., LTD., Tokyo, Japan). Briefly, 0.2ml of serum or 0.2 ml of standard secretin solution were added to 1.0 ml of rabbit anti-secretin serum against pure porcine synthetic secretin; the mixture was stirred and incubated at 4°C for 4 days. Then 0.1 ml of ^{125}I(Tyr^1) secretin was added to each tube and incubated at 4°C for 1 day. Precipitations of the soluble secretin antibody complex were achieved by adding 0.1ml of a second antibody generated in sheep against rabbit gamma-globulin. Each tube was stirred and incubated at 4°C for another day. Then the tubes were centrifuged at 2,000 g for 30 minutes at 4°C. The supernatant of each tube was aspirated, and the precipitated samples were counted in an automatic gamma spectrometer (Nuclear Chicago, Ilinois, USA). The sensitivity of this assay was 50 pg of secretin per test tube. The cross-reaction rates with other hormones such as CCK-PZ, VIP, GH, ACTH, Glucagon, TSH were reported to be below 0.003%, whereas the reaction rate of secretin was 100%.
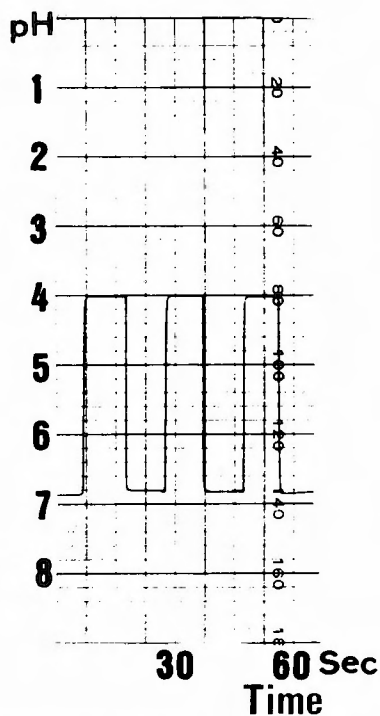
Fig. 3.   The obtained response time for a shift between pH 6.86 and pH 4.01.

Results

1)   Changes of duodenal bulbar pH

Duodenal bulbar pH changes were grossly classified into three phases (Fig. 4).   Immediately after meal, the pH transiently became about 0.2 to 1.0 pH units more alkaline than the highest fasting pH values.   The onsets of the pH shift started within 30 seconds after the beginning of feeding, and the peaks of the shift were achieved within 5 minutes.   The distortion toward greater alkalinity was not affected by administration of atropine sulphate 0.5 mg intravenously, but similar distortions were observed by administration of epinephrine 0.1 mg intravenously (Fig. 5).   The postprandial gradual decline of pH values started between 5 and 45 minutes after feeding (average of 18.7 minutes).   During the first 5 to 7 hours (mean=6.0 hours, sd=0.88 hours) after the meal, duodenal bulbar pH showed slow cyclic changes between pH 6.4 and pH 3.2; this was designated the postprandial phase (stable weak acid phase).

During the next 5 to 7 hours (mean=7.5 hours, sd=2.0 hours), bulbar acidity fluctuated slowly and abruptly between pH 7.0 and pH 2.0; this phase was designated the transitional phase (moderate fluctuating acid phase).

Fourteen hours after meal (mean=13.6 hours, sd=2.28 hours), pH change patterns could be divided into two subphases, the neutral or alkaline subphase and the wide fluctuating strong acid subphase.   In the neutral or alkaline subphase, pH values were stabilized between pH 6.8 and pH 7.4.   The duration of this subphase ranged from 7 minutes to 225 minutes, with an average of 60 minutes.   In the wide fluctuating strong acid subphase, pH fluctuated between
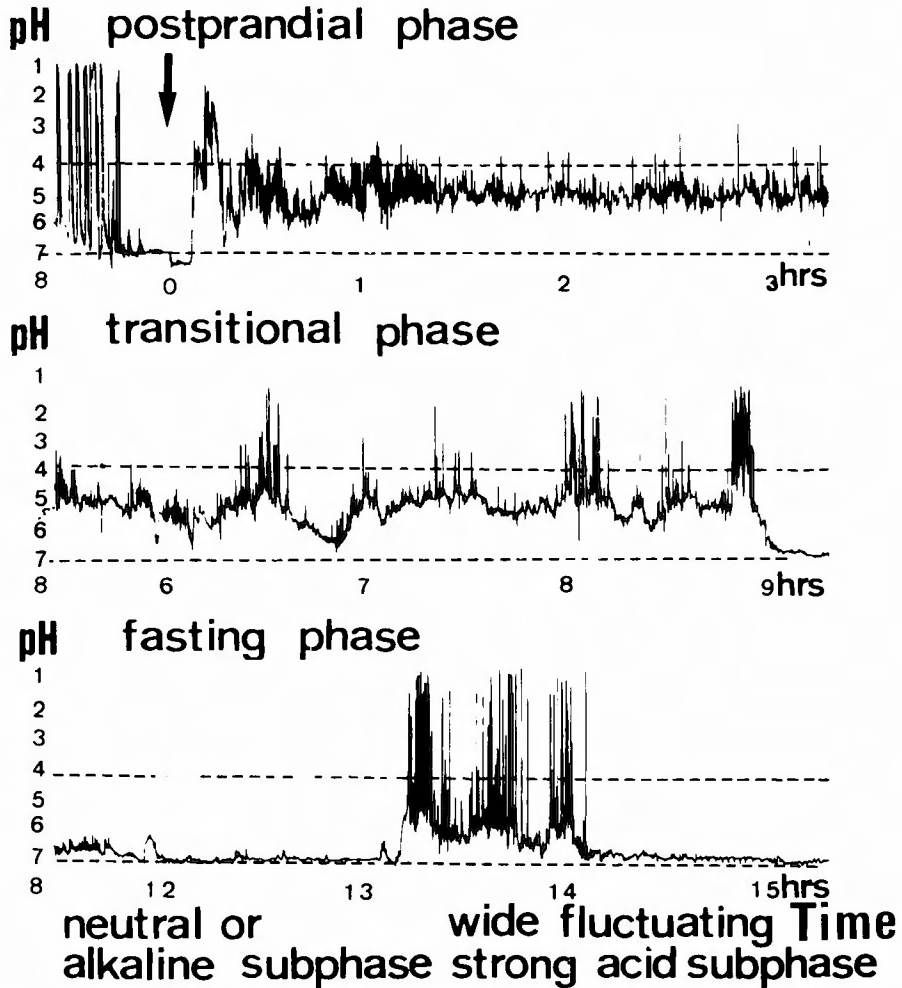
Fig. 4. Changes in duodenal bulbar pH. (Top) The arrow indicates feeding. Immediate rise in pH after feeding was noted and pH suddenly declined at 8 minutes after feeding. pH showed cyclic changes between pH 3.2 and pH 6.0. (Middle) pH recorded between 6 and 9 hours after feeding. pH ranged between pH 6.8 and pH 1.8. (Bottom) The changes during this phase were divided into two subphases. pH was stable in the neutral or alkaline phase, but changed between pH 7.0 and pH 0.8 in the wide fluctuating strong acid subphase.

pH 7.2 and pH 1.2 sometimes abruptly and sometimes slowly. The duration of this subphase ranged from 7 minutes to 135 minutes, with an average of 44 minutes. The frequencies of the fluctuation ranged from 4 per minute to 1 per 4 minutes. The neutral or alkaline subphase and the wide fluctuating strong acid subphase were seen alternately until the next meal (Fig. 6). This phase was designated the fasting phase.

2) Fasting secretin levels

In dogs fasting for more than 16 hours, duodenal bulbar pH was recorded simultaneously with blood aspiration through the venous cannula for the radioimmunoassay of secretin. The secretin levels were stabilized in the neutral or alkaline subphase. The secretin levels were
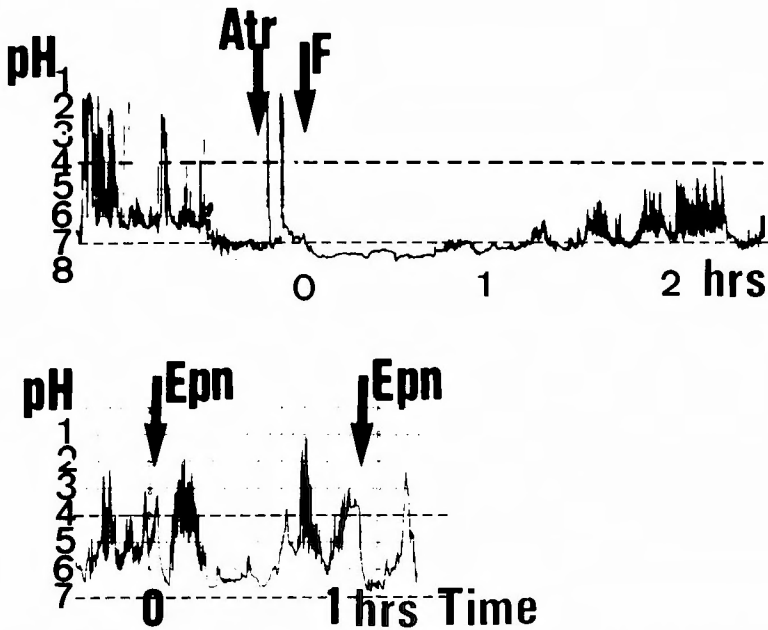
Fig. 5. A recording of duodenal bulbar pH after administration of atropine sulphate 0.5 mg intravenously. (Above) Note the rise in pH after the beginning of feeding. The onset of pH shift to more alkaline started within 30 seconds and the peak of pH shift was achieved within 3 minutes. (Below) A recording of duodenal bulbar pH after administration of epinephrine 0.2 mg intravenously. A similar rise in pH was observed in the fasting phase.

significantly higher in the wide fluctuating strong acid subphase than in the neutral or alkaline subphase ($p < 0.001$) (Fig. 7). When the duration of pH fluctuation to strong acid was too short, no increase in serum secretin levels was observed.

Discussion

The technique used in this study permits the continuous measuring of duodenal bulbar pH. Its flexibility and thinness enables us to measure intraluminal pH at any site of the alimentary tract. It can be easily inserted orally through enterostomy, and pH of the optimal site can be easily measured. The sensor tip is constructed so that it never come in contact with the mucous membrane. BIRCHER[4] stressed that a glass membrane electrode should be protected by a guard in order to prevent contact with mucous membrane. However, guarding the tip has some disadvantages. According to RHODES[18] the shielding around the tip became clogged with mucus or food preventing the recording of wide pH fluctuations. Our instrument avoids these problems. The response time is short enough to detect any change in intraduodenal pH. Using a glass membrane electrode, ITOH[12] reported that the response time for a shift from pH 7 to pH 4 was below 3 seconds but it took 5 to 10 seconds for shift from pH 4 to pH 7. In contrast, the response time of our sensor is surprizingly short. In addition, the sensor is so stable that the deviations of pH values over 24 hours are below 0.2 pH units.
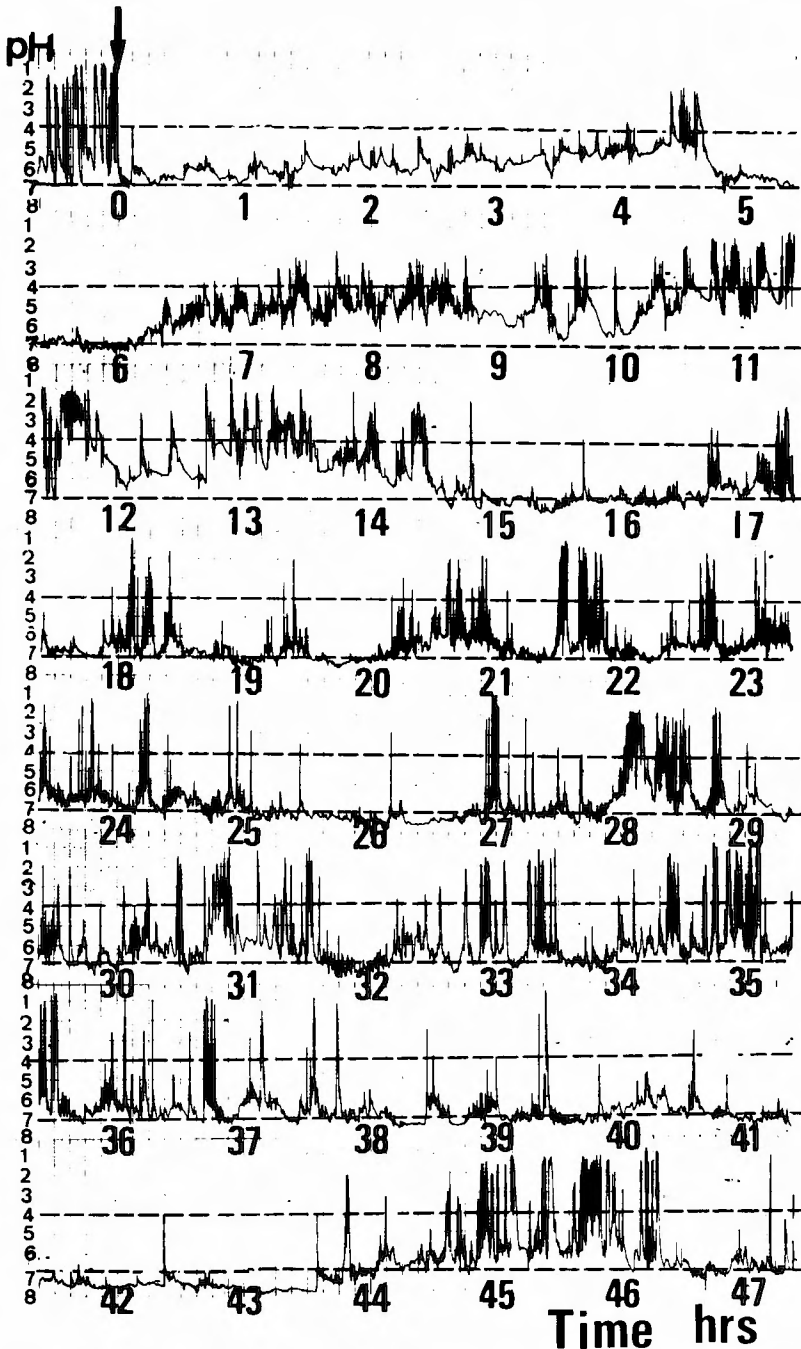
Fig. 6. A 48-hour recording of duodenal bulbar pH. Fasting phase continued until next meal. Alternations were seen between the neutral or alkaline subphase and the wide fluctuating strong acid subphase.

Bulbar pH shift toward greater alkalinity, about 0.2 to 1.0 pH units immediately after the meal was observed. The onsets of the shift occurred shortly after the meal. No precedence of low pH, enough to secrete secretin after meal, was required. The administration of atropine
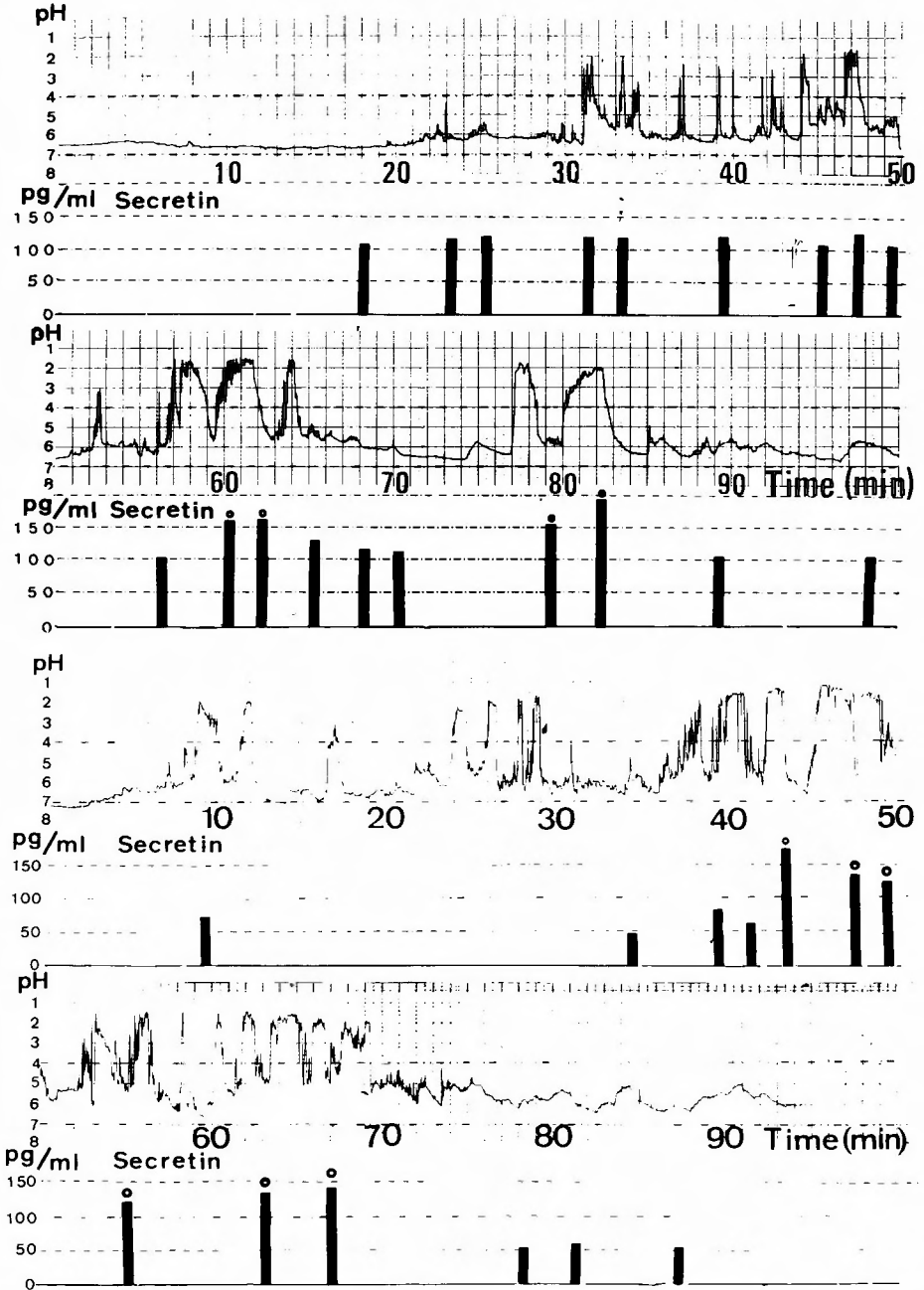
Fig. 7. The fasting secretin levels. The plasma secretin levels were stabilized in the neutral or alkaline subphase but they increased to significantly higher levels in the wide fluctuating strong acid subphase. ○ indicates stastical significance (P < 0.001).

sulphate 0.5 mg intravenously was not able to block the shift.   The administration of epinephrine resulted in the similar shift of bulbar pH.   We also found that licking of a duodenal cannula caused a similar shift (unpublished data).   These findings suggested that duodenal bulbar neutralization in the early stages after a meal was regulated by adrenergic fibers.   Further investigations concerning to this problem will be reported in the near future.

pH of the duodenal bulb after homogenized liquid meat meal presented less than 3 pH unit cyclic variations, and pH during this phase rarely decreased below pH 3.2.   The lowest pH values during this phase (pH 3.2) were almost the same as the pH value previously reported by BROOKS[5,6].   Since the pH threshold for secretin release was reported to be between pH 2 and pH 3 by FAHRENKRUG[9] and LLANOS[15], the relationship between the secretin release and duodenal bulbar pH during this phase required further exploration.

The variations of the duodenal pH in the fasting phase was reported to be within one pH unit in dogs[13], pigs[7] and humans[19,20].   Measuring of pH was done in the second portion of the duodenum in these series.   pH obtained in the fasting state in our series showed two different patterns, as described above, a neutral or alkaline subphase and a wide fluctuating strong acid subphase.   Our observations coincided with the findings reported by others[1,8,14,16,17]; pH was measured in the duodenal bulb in these series.   It is necessary to measure intraluminal pH of the duodenal bulb to observe wide fluctuations in the fasting phase, because there is a steep gradient of pH between the base of the duodenal bulb and the second portion of the duodenum[14], and because the postbulbar duodenal pH is higher and more stable than pH of the duodenal bulb[21].

The observation of the high acidity of intraluminal pH of the duodenal bulb suggested changes in the secretin levels in the fasting phase.   Although the secretin-secretion in the post-prandial phase has been a subject of controversy for a long time, almost no attention has been paid to what the standard secretin level in the fasting state is.   In our series secretin levels were found to be stable in the neutral or alkaline subphase, but they changed to statistically higher levels in the wide fluctuating strong acid subphase.   Our findings coincided with those reported by SCHAFFALITZKY de MUCKADELL[20].   In his work, when a bolus of acid 2.5–10.0 ml–0.1 N HCl was injected into the duodenal bulb, the plasma secretin increased in relation to duodenal bulbar pH.   The bulbar pH changes after injection of acid into the duodenal bulb resembled more closely the patterns in the wide fluctuating strong acid subphase of the fasting phase than the patterns in the postprandial phase.

Thus, it is important to measure duodenal bulbar pH simultaneously when blood is aspirated for secretin determination, and the secretin standard levels should be defined as the levels when duodenal bular pH is in the neutral or alkaline subphase.

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和文抄録

成犬における無麻酔下の十二指腸球部 pH の24時間測定と
飢餓時セレクチンの分泌動態

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中　元　光　一

飢餓時のセクレチン分泌動態を知る目的で，雑種成犬を用い，無麻酔下に十二指腸球部 pH を24時間測定し，且つ飢餓時の球部 pH とセクレチン血中濃度との比較検討を行なった．十二指腸球部 pH の日内変動はⅢ期に分類できた．

第Ⅰ期(飽食期)：食後5〜7時間で pH 6.4〜3.6 の弱酸性安定期．

第Ⅱ期(移行期)・第Ⅰ期に続く5〜9時間で pH 7〜3を緩慢に変動．

第Ⅲ期(飢餓期)：食後11〜16時間以降で，さらに2相に分けられる．

a 相：pH 7前後で安定し殆んど変化なし．

b 相：pH 7 と pH 1 前後との間を，時には急激に，時には緩慢に変動する．

すなわち，飢餓期には，十二指腸球部 pH は，中性安定期と強酸性変動期との2相があることが判明した．飢餓期のこの異なった相の時期に採血し，セクレチン濃度を測定して比較検討したが，強酸性変動期にはセクレチン濃度が有意に上昇した．セクレチン分泌動態を検討するときは，十二指腸球部 pH を同時に測定することが，不可欠と思われる．