THE BIOASSAY OF SECRETIN IN THE RAT

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In all methods for the assay of secretin now in use, the hormone is intravenously injection into the experimental animal and the response of the pancreas is recorded. The animals used have been dogs, cats, rabbits and rats (1). The rat method was elaborated in 1957 by Love (2), and Svatos and Jelinek (3). Although this rat method was shown impractical by Lin and Alphin in consequence of their comparative experiments between rats and dogs (4), it has been used by some groups (5–7). The technique of the rat method has been recently reinvestigated by Heatley and he shows that secretin can be assayed in rats (8).

For several years we have worked up the purification and economic production of secretin. In the course of this work the necessity arose for developing a simple method for the assay of secretin content in large numbers of preparations. Mutt and Söderberg found that if certain precautions were taken, anesthetized cats could be used for the assay of secretin for several days, thereby eliminating the necessity of time-consuming daily operations (9). But their valuable method which can be used to follow the activity of secretin in the course of purification is not suitable for determining precisely the quantity of secretin in preparations. Except Dorchester and Haist who used rabbits, none of the experiments have been statistically done for the estimation of secretin potencies (10).

We chose rats for the assay of secretin, because we could easily obtain the rats whose strain, age and body weight were nearly similar. The following facts were confirmed. Secretin could be satisfactorily assayed in anesthetized rats by the measurement of the volume of pancreatic juice secreted. The technique of the rat method was very simple, and if the twin crossover design which was used to determine various hormone potencies was applied, the expected standard error of the estimate was 10 to 15 percent. Furthermore secretin could be also assayed if the bicarbonate output in the pancreatic juice was determined, which changed linearly with the log doses of secretin.

Because of the lack of the international unit and a stable standard of secretin, there is no precise comparison between the potencies of the various units reported such as rat, dog, cat and Clinical unit yet (1, 5). The variation in the secretin potency between two brands of commercial secretins has been recently found to differ significantly (11). The relative potency between Crick, Harper and Raper unit (12) and Swedish Clinical unit (13), both of which were used in the clinical diagnosis for the pancreatic disorders, was determined by this rat method. The potency of 1 Clinical unit was 2 to 4 times as strong as that of the former.
MATERIALS AND METHODS

1) Secretin: The following three preparations were used.

Boots secretin which was obtained from Boots Pure Drug Company Ltd. Nottingham, England.

Highly purified and pure natural secretin, which were obtained Gastrointestinal Hormone Laboratory, Karolinska Institute, Stockholm, Sweden Batch No. 16642, 16921.

Secretin extracts, which were extracted from the fresh porcine duodenum and partially purified.

2) Rats used: Male Wistar-Imamichi rats (weighing 300-500 g) were purchased from the Institute of Animal Reproduction, Omiyashi, Saitamaken.

3) Anesthetics: Urethane 1.42 g per kg and sodium pentobarbital 70 mg per kg body weight were intramuscularly or intraperitoneally injected. This urethane dose usually maintained rats at a suitable level of anesthesia for 10 to 15 hours, but sodium pentobarbital was injected 1 mg per rat every hour after a few hours of the completion of the operation.

4) Operation: The pure pancreatic juice of rat was collected according to the method described by Love (2). In some of the rats the pylorus was not ligated to determine the effects of gastric juice on the pancreatic secretion.

5) Measurements of the volume of pancreatic juice and the bicarbonate concentration: The rate of pancreatic secretion by secretin injected was determined by measuring the distance filled by the juice in the polyethylene tube (1 cm equal 3.42 μl) at regular intervals which was then washed out into 20 ml messflask, added a known excess of N/100 hydrochloric acid and distilled water to 20 ml. The mixture was then heated for 5 minutes in boiling water bath. The bicarbonate secreted was back titrated with N/20 sodium hydroxide to the pH of the distilled water used, usually 5.6 ±0.2, by pH Stat, Radiometer, Copenhagen, Denmark.

6) Assay procedure: The rats were fasted for 24 hours and the body temperature was maintained at 34 to 36° by 60 W lamp. Pancreatic secretion was ascertained by intravenous injection of 2 or 4 CHR units (Crick, Harper and Raper units) of secretin after the operation and stabilized by the one more same dose of secretin after 30 minutes of the first injection. Secretin response was measured as the volume of the pancreatic juice during 30 minutes after the injection, because the pancreatic response of 2 to 4 CHR units was ceased at 30 to 40 minutes after the administration. The basal secretion was measured as the volume during 15 minutes before the injection. For the quantitative assay the twin crossover design was usually employed. The differences of secretion between high and low dose were calculated. The high dose of the standard secretin was 4 CHR units and the low one was a half of the high. The ratio of the high to the low dose of the standard and the unknown samples was two. The qualitative potencies of many samples were determined by the comparison between 30 minutes responses to unknown samples and those to known standards which were interspersed at intervals of 2 or 3 hours.
RESULTS

1. Log doses of secretin and pancreatic responses

A linear regression line of the pancreatic secretion on the log doses of secretin was obtained by the method of least squares. By analysis of variance this regression line was highly significant regardless of the ligature of the pylorus. But in the rats, the pylorus of which was not ligated, the variance of the differences from the regression line was also significant. On the other hand, in the pylorus-ligated rats this variance was not significant (Fig. 1, Table 1). From these results the pylorus-ligated rats were suitable for the assay of secretin, because their pancreatic secretion was stable, although the slope of their regression line was gentle.

2. Variation of responses during the assay

Since in the twin crossover design rats were twice injected high and low doses of the standard secretin and those of the unknown preparation respectively, the first pancreatic response to the standard secretin was compared with the second one to the same dose of the standard (Table 2). These figures were consecutively chosen from animals in routine assays. In the rats, the pylorus of which was not ligated, the total pancreatic secretion

![Diagram](Image)
TABLE 1. Analysis of variance.

| Factor                             | SS     | Df | Ms   | VR   |
|------------------------------------|--------|----|------|------|
| Linear regression                  | 12415.74 | 1  | 12415.74 | 76.08** |
| Difference from regression         | 491.35  | 2  | 245.68 | 1.51 |
| Dose                               | 12907.09 | 3  | 4302.37 | 26.36** |
| Error                              | 14850.02 | 91 | 163.19 |      |
| Total                              | 27757.11 | 94 |       |      |

A linear regression line: \( Y = 13.8X + 20.1 \)

** Significant at 0.01 level.

Abbreviations used in this report. SS: Sum of squares, Ms: mean of squares, Df: degrees of freedom, VR: variance ratio, N: number of experiments.

| Factor                             | SS     | Df | Ms   | VR   |
|------------------------------------|--------|----|------|------|
| Linear regression                  | 53335.97 | 1  | 53335.97 | 161.63** |
| Difference from regression         | 3185.69 | 2  | 1592.85 | 4.83** |
| Dose                               | 56521.66 | 3  | 18840.55 | 57.09** |
| Error                              | 53457.01 | 162 | 329.98 |      |
| Total                              | 109978.67 | 165 |       |      |

** Significant at 0.01 level.

A linear regression line: \( Y = 24.4X + 8.9 \).

TABLE 2. Effect of repeated doses of standard secretin on the pancreatic secretion at the twin crossover design.

(A) Normal pylorus rats. Mean of body weight 420 g (N = 71)

| Secretin dose | First injection | Second injection |
|---------------|-----------------|------------------|
|               | Juice vol:30 min| Increased vol:30 min** | Juice vol:30 min| Increased vol:30 min** |
| 4 CHR units*  | 94.63±2.80 pl   | 80.64±2.36 pl     | 102.46±3.04 pl  | 76.20±2.16 pl |
| 2 CHR units   | 76.23±2.03      | 58.62±1.65        | 82.59±2.65      | 58.20±1.74   |

(B) Pylorus-ligated rats. Mean of body weight 420 g (N = 33)

| Secretin dose | First injection | Second injection |
|---------------|-----------------|------------------|
|               | Juice vol:30 min| Increased vol:30 min | Juice vol:30 min| Increased vol:30 min|
| 4 CHR units   | 76.90±2.61 pl   | 60.82±2.42 pl     | 75.88±2.32 pl   | 56.25±1.72 pl |
| 2 CHR units   | 65.27±2.68      | 47.52±2.39        | 65.01±1.93      | 45.23±1.51   |

* Crick, Harper and Raper unit.

** Total 30 minute secretion minus double of basal 15 minute secretion before injection.

during 30 minutes tended to increase gradually in the latter half of the assay. On the other hand, the pancreatic secretion in response to secretin, which was defined the total secretion during 30 minutes minus the basal one measured before the administration, tended to decrease in the latter half of the assay, i.e. the basal secretion increased. This tendency was conspicuous in the rats with normal pylorus. In the usual assay it was capable
to avoid this tendency, if the control secretion time lasted until the rate of the basal secretion after the response decreased to the same rate as that of the preceding one.

3. Relation between rat body weight and secretin response

How the size of the rats used affected on the bioassay of secretin was studied. Table 3 shows the comparison between secretin responses in large and small rats. The large rats displayed a bigger response than the small, when the same dose was administered into the both animal groups. But the volume of pancreatic juice per 100 g body weight and the slope of the dose response line of the both animal groups were not significantly different.

| Rat body weight | Average Range | At random | Large | Small |
|-----------------|---------------|-----------|-------|-------|
|                 |               | 420 g     | 330~500 g | 480 g | 460~540 g | 360 g | 330~590 g |
| Number of rats  |               | 37        | 10    | 10    |
| pl/100 g body weight/30 min | 4 CHR units+ | 14.20 ± 0.4 pl | 13.71 ± 0.67 pl | 15.34 ± 0.82 pl |
|                 | 2 CHR units  | 11.05 ± 0.40 pl | 10.75 ± 0.80 pl | 11.43 ± 0.59 pl |
|                 | 4 CHR units  | 59.20 ± 1.71 pl | 65.32 ± 2.74** pl | 55.06 ± 2.87** pl |
|                 | 2 CHR units  | 46.17 ± 1.68 pl | 50.96 ± 3.45** pl | 40.70 ± 2.02** pl |

+ Crick, Harper and Raper unit.

** The differences of secretion between large and small rats are significant: P = 0.05.

4. The twin crossover design for bioassay and confidence limits of the estimates

Having been established the basic linear relationship between the pancreatic responses and the secretin doses, model assays, in which one of the three different dilutions of the same secretin solution was served as the Standard and the other two were served as the Unknowns, were carried out so that the confidence limits of the estimates were clarified. In the usual procedure of the assay of unknown preparations, the approximate potency of the Unknown was first determined, then it was administered in doses nearly equal to the doses of the Standard employed. It is necessary that the dose-ratio of the low to the high Unknown is equal to that of the low to the high Standard. In this report the ratio was two and the Standard solution had a potency of 20 CHR units per ml, while the Test solutions had a potency of 15, 20 and 25 CHR units per ml respectively. Each assay was carried out by three rats (Table 4). It can be concluded from the results shown in Table 4 that the rat method is quite suitable for the assay of secretin as well as the dog and the cat method, and that the errors of the estimates are 10 to 15% and the estimates are quite reproducible.

5. Correlation between the Crick, Harper and Raper unit and the Swedish Clinical Unit (GIH unit)

To express the secretin potency CHR unit and the Clinical unit have been widely used. The unit of potency employed for secretin by Boots is that defined by Crick, Harper and Raper (12) and the unit of potency employed Gastrointestinal Hormone Laboratory (GIH) is the Clinical unit of Hammarsten, Ågren and Lagerlöf (16), the standard of which has been able to be obtained as GIH unit from Karolinska Institute, Stockholm, Sweden.
### Table 4. Confidence limits of the estimated secretin potency by twin crossover design (pylorus-ligated rats).

Standard solution: 20 CHR units/ml.
S\text{H}: 4 CHR units/0.2 ml i.v., S\text{L}: 2 CHR units/0.1 ml i.v.

(A) Test solution 20 CHR units/ml

| Rat No. | S\text{H}-T\text{L} | T\text{H}-S\text{L} | T\text{H}-S\text{L} | S\text{H}-T\text{L} |
|---------|---------------------|---------------------|---------------------|---------------------|
| 201     | 29.1 p\mu l         | 10.3 p\mu l         | 23.3 p\mu l         | 18.5 p\mu l        |
| 202     | 31.1                | 16.1                | 29.8                | 8.6                |
| 203     | 22.9                | 2.1                 | 21.1                | 10.3               |

Estimate: Standard 1 unit = Test 0.94 units

| T\text{H} 4 units | 3.8 (3.342) ** |
| T\text{L} 2 units | 1.9 (1.742) |

* The difference of secretin response during 30 minutes between test and standard solution after the administration.

** 95% confidence limits.

(B) Test solution 25 CHR units/ml

| Rat No. | S\text{H}-T\text{L} | T\text{H}-S\text{L} | T\text{H}-S\text{L} | S\text{H}-T\text{L} |
|---------|---------------------|---------------------|---------------------|---------------------|
| 222     | 11.6 p\mu l         | 7.5 p\mu l         | 9.6 p\mu l         | 5.1 p\mu l         |
| 223     | 20.2                | 31.8                | 25.7                | 9.6                |
| 224     | 3.4                 | 25.7                | 23.6                | 6.2                |

Estimate: Standard 1 unit = Test 1.3 units

| T\text{H} 5 units | 5.2 (4.26.8) |
| T\text{L} 2.5 units | 2.6 (2.13.4) |

(C) Test solution 15 CHR units/ml

| Rat No. | S\text{H}-T\text{L} | T\text{H}-S\text{L} | T\text{H}-S\text{L} | S\text{H}-T\text{L} |
|---------|---------------------|---------------------|---------------------|---------------------|
| 226     | 29.1 p\mu l         | 5.8 p\mu l         | 9.9 p\mu l         | 19.2 p\mu l        |
| 227     | 20.9                | 18.1                | 13.7                | 15.1               |
| 229     | 16.4                | 7.9                 | 7.2                 | 18.1               |

Estimate: Standard 1 unit = Test 0.8 units

| T\text{H} 3 units | 3.2 units (2.73.8) |
| T\text{L} 1.5 units | 1.6 units (1.31.9) |

Although the usual recommended dose of each of the both secretin units is 1 unit per kg body weight in the clinical diagnosis of the pancreatic disorders, the potency of the both units has been known not to be identical (1, 11). Healtey has also suggested that the potency of the GIH unit itself after 1967 was several times greater than that used earlier (8).

When the potency of CHR unit had been compared with GIH unit, GIH secretin batch No. 16642, at 1967 by this rat method, 1 GIH unit was found to be equivalent to 2 CHR units (Table 5A). The result described here was in good agreement with the value indirectly estimated by Jorpes and Mutt (1). "S\text{H}-T\text{L}" in Table 5 means the difference of the pancreatic responses during 30 minutes between the dose of 4 CHR units of the standard and that of 1 GIH unit of the test secretin. "T\text{H}-S\text{L}" means the difference between 2 GIH units of the test and 2 CHR units of the standard secretin. The order of the admin-
TABLE 5. Correlation of Crick, Harper and Raper unit and Swedish Clinical unit.

A) Standard solution: 20 CHR units/ml.  
SH 4 units/0.2 ml/Rat, SL 2 units/0.1 ml/Rat.  
Test solution: GIH secretin batch No. 16642, 10 GIH units/ml.  
T_h 2 units/0.2 ml/Rat, T_l 1 unit/0.1 ml/Rat.

| Rat No. | S_h-T_l | T_h-S_l | T_h-S_l | S_h-T_l |
|---------|---------|---------|---------|---------|
| R-157   | 20.52 ml| 6.16 ml | 4.79 ml | 19.49 ml|
| R-158   | 13.00   | 30.78   | 17.10   | 12.39   |
| R-180   | 23.60   | 25.31   | 18.47   | 12.74   |
| R-181   | 14.02   | 8.21    | 13.34   | 5.47    |

1 Clinical unit = 2.01 CHR units 95% Conf. limit: 1.70–2.40.

B) Standard solution: 20 CHR units/ml.  
SH 4 units/0.2 ml/Rat, SL 2 units/0.1 ml/Rat.  
Test solution: GIH secretion batch No. 16921, 5 GIH units/ml.  
T_h 1 unit/0.2 ml/Rat, T_l 0.5 unit/0.1 ml/Rat.

| Rat No. | S_h-T_l | T_h-S_l | T_h-S_l | S_h-T_l |
|---------|---------|---------|---------|---------|
| 254     | 12.65 ml| 13.00 ml| 6.84 ml | 13.00 ml|
| 255     | 20.52   | 24.28   | 16.75   | 9.23    |
| 256     | 14.02   | 29.75   | 20.86   | 21.55   |
| 261     | 26.68   | 13.34   | 10.26   | 19.84   |

1 Clinical unit = 3.98 CHR units 95% Conf. limit: (3.44–4.59).

The administration of two pairs was randomly injected and the order within the pair was inverted at the latter half. Since GIH unit itself was found to be ambiguous as aforesaid, CHR unit was again compared with new GIH secretin, batch No. 16921, at 1969.

Table 5B shows that 1 GIH unit is equivalent to 4 CHR units. These results indicated that the potencies of the both units were quite different, i.e. the potency of 1 Swedish Clinical unit was 2 to 4 times greater than that of 1 CHR unit. The value obtained here was not in agreement with the observation of Stening, Vagne and Grossman who showed that the potency of 1 GIH unit was 8 to 9 times greater than that of 1 CHR unit (11). But this discrepancy, at least in part, may be attributed to the difference of the assay method between the anesthetized rats and the conscious dogs. Furthermore it seems

FIG. 2. Relationship between log dose of secretin and the amount of sodium bicarbonate secreted during 45 minutes after the injection at 8 rats with the normal pylorus. A linear regression line determined by the method of least squares yield the equation: \( Y = 1.88X + 2.62 \).

This regression line is highly significant from the variances (\( P < 0.01 \)).
likely that the potency of 1 Swedish Clinical unit of the batch No. 16921 was twice as active as that of the batch No. 16642 in harmony with the results of Healtey (8).

6. The amount of sodium bicarbonate secreted and graded doses of secretin

As shown in Fig. 2 and Table 6, the amount of sodium bicarbonate secreted in the pancreatic juice increases proportionally to the log doses of secretin injected. A linear regression line determined by the method of least squares was highly significant from the variances (P<0.01). Therefore the potency of secretin could be estimated by the titration of the bicarbonate in the pancreatic juice of rats. On the contrary, the mean concentration of bicarbonate in the juice produced by eight rats was nearly constant in spite of the administration of the graded doses of secretin. This observation was inconsistent with the result of Heatley (8), which showed that the amount and the concentration of bicarbonate were increased after giving secretin.

DISCUSSION

The techniques of performing the assay of secretin in rats have varied on a few points with the reports. One of them is whether the pylorus of the rat is ligated or not. Debray et al used the rats, the pylorus of which was not ligated (5). On the other hand, Svatos and Jelinek (3), Love (2), and Heatley (8) used pylorus-ligated rats. In addition, the limited ranges of the linear relationship between the doses of secretin and the pancreatic responses were quite different, because the potency and the unit of secretin used were not identical. These limited ranges previously reported are calculated as follows in terms of Hammersten Cat Unit (HCU) (13, 14), which has been well defined in relation to the other units suggested.

| Secretin dose (Crick, Harper & Raper units) | 1   | 2   | 4   | 8   |
|--------------------------------------------|-----|-----|-----|-----|
| NaHCO₃ output meq/45 min                    | 4.73 ± 0.35 | 6.13 ± 0.97 | 8.03 ± 1.20 | 10.35 ± 1.01 |
| NaHCO₃ concentration meq/L                  | 56.7 ± 4.8  | 58.1 ± 2.6  | 57.1 ± 2.7  | 61.5 ± 2.7  |
| Number of rats                              | 8   | 8   | 8   | 8   |

The results were expressed as the averages ± standard errors obtained two responses injected in the rats with no ligation of pylorus.

We have initially investigated: what change shall occur if the pylorus of the rat is ligated? The ligation of the pylorus tended to decrease the pancreatic secretion in doses of more than 2 CHR units per rat, but in doses of secretin below 2 CHR units there was no difference between the both ligated and normal groups. It seems likely that the increase in the pancreatic secretion in the rats with the normal pylorus especially in high
doses is due to the contaminations. Secretin preparation used here may be contaminated by other physiologically active substances, for example, Cholecystokinin-Pancreozymin and histamine which stimulate gastric secretion. They accelerate release of the endogenous secretin. Secretin used as the standard in this experiment was extracted from the fresh porcine doudenum and partially purified to the grade of 8.3 CHR units per mg. Linearity of the responses were seen in both groups of rats in a dose range of 0.5 to 8 CHR units, i.e. 5 to 80 HCU, per rat. However, as described previously in many reports, the slope varied from animal to animal, especially in rats whose pylori were not ligated. The variances from the regression line were so significantly different that the rats with the normal pylorus were not suitable for the assay of secretin. On the other hand, in the pylorus-ligated rats the variances are so small that secretin can be conveniently assayed. When the potency of secretin was determined in rats, the assay design used was to give alternating “high” and “low” doses of the Standard, interspersed with the same or different doses of the Unknown. For most of the works previously reported the potency of the Unknown was read off from the line joining the response to “high” and “low” Standards on ordinary graph paper (2, 3, 8). In these designs the confidence limits of the estimate are quite ambiguous.

The twin crossover design was used in this paper.

“High” (4 CHR units which were equivalent to 40 HCU) and “low” (one half of “high”) doses of the standard were used. It was demanded that the ratio between “high” and “low” doses were as small as possible in the twin crossover design, because the parallelism between the standard and the test line could not be assayed. Although Lin and Alphin pointed out that the rats receiving pentobarbital did not respond to single intravenous secretin (4), the present results showed that the rats anesthetized with pentobarbital responded to the graded dose of secretin. However, pentobarbital did not last the anesthesized state so long that urethane was the best anesthetic for rat in harmony with the many previous observations. As it was confirmed that the response of the conscious rat was many times greater than that of the anesthetized rat (4), the deepness of anesthesia was one of the important factors which affected the assay of secretin. Therefore, the twin crossover design in which the responses of secretin are repeated in the same individual has the advantage of making the errors of the estimated potency smaller than the usual four points design. The experiments with a few hundred unknown samples supported this concept and the errors of the estimates using 2 to 4 rats were 10 to 15% (Table 4 ABC). The response to secretin must be calculated from the basal secretion and the amount of the increased one produced by the administration of secretin, but the results in Table 2 indicate that the extra secretion during 30 minutes after the administration of both “high” and “low” doses is more reproducible than that of the increased secretion minus the basal one. If the secretion during 30 minutes after the injection as well as the following control secretion during 15 minutes would be observed, namely, the rats would be administered secretin every 45 minutes, it should be in need of long time to assay of one unknown sample. Since the response to secretin weakens after 20 minutes of the injection, one can regard the response as the secretion during 25 minutes after the injection. Then in all
routine assays the responses to secretin measured as the total secretion during 25 minutes after the administration. Determined the secretion during 25 minutes as well as the following control during 10 minutes, secretin can be injected every 35 minutes so that one can save the time for the assay beyond one hour. If the 10 minutes control secretion after the 25 minutes response is more than one and a half times greater than that before the injection, it is necessary to wait further 10 minutes before the next administration.

Heatley's report showed that the difference in the responses of rats was related to sex rather than to size (8). As shown in Table 3, the differences in the responses among the various body weights of rats do not participate with the assay of secretin. Other factors which affect the assay are fluctuations and taphyphylaxis. Either gradually or abruptly, the response of the rat to the same dose of secretin sometimes varied during the assay. Heatley stated that it was impossible to relate fluctuations in response to any factors, such as diet, age, sex or body weight of the rat, plane of anesthesia, body temperature, or the presence of noxious agents in the solutions assayed. But some fluctuations might be due to the "distortion effect" brought into operation, which was eliminated by the perfect cannulation technique (8, 20). A gradual change of the response to the same dose of secretin is not important for our assay, because the potency of secretin in the twin crossover design must be calculated from the differences between the test and the standard responses in series. An abrupt change due to fluctuations vitiates the assay, but some of them can be excluded if the concentration of protein secreted in the pancreatic juice is sufficiently lowered, and the pancreatic juice is faintly aspirated. For this purpose, 4 CHR units were twice administered for the wash out of proteins from the duct system after the completion of the operation, and the calibration tube was situated 10 to 15 cm lower than the back of supine rats.

Heatley also reported that even very crude preparations did not make the rat pancreas refractory to secretin stimulation, except the first administration gave a bigger response (8). In the same and subsequent report, he indicated that pure cholecystokinin-pancreozymin, which was one of gastrointestinal hormones occurred in the duodenum, was a strong stimulant of the pancreatic juice and the alkali secretion, and that the potency of secretin and cholecystokinin-pancreozymin could be assayed simultaneously (20). He did not mention a possible interaction between secretin and cholecystokinin-pancreozymin when the both were administered simultaneously. It, however, was observed that even a little amount of cholecystokinin-pancreozymin contaminated in the secretin preparation should potentiate to increase the volume of the pancreatic juice secreted by secretin, and that this potentiation effect of cholecystokinin-pancreozymin was progressively diminished by repeated injections of the same secretin preparation. Thus, coupled with the fact that there was a marked synergism between secretin and cholecystokinin-pancreozymin in cats (21), it would be concluded that the net potency of secretin in the preparation contained unknown amounts of cholecystokinin-pancreozymin could not be principally determined. But further work is required to clarify this problem, because pure cholecystokinin-pancreozymin is not easily obtainable.
It has been well known that secretin is a stimulant of the pancreatic secretion both in its volume and sodium bicarbonate, and besides the amount of bicarbonate secreted in the pancreatic juice against the graded doses of secretin is linear in certain limits as well in cats (15) as in men (16). It was also confirmed in this paper that in rats too the amount of bicarbonate was secreted almost stoichiometrically proportional to the dose of secretin injected, therefore, secretin potency could be estimated by the titration of the sodium bicarbonate in the pancreatic juice of rats.

Taking for the preparation of each rat used in the assay less than 20 minutes, one can operate several rats in a day and can assay one or two samples simultaneously. Before an accurate assay is done, its approximate potency is needed to estimate. One rat can permit several samples to be estimated its qualitative potencies. Thus, these results shown here support the suggestion that the rat is suitable for the assay of secretin as well as dog and cat, because rat has the advantages of being easily obtainable and the operation technique is simple.

The potency of secretin used to express in various units such as Hammersten cat unit (13, 14), Ivy dog unit (17, 18), Ivy cat unit (18), Lagelof clinical unit (GIH unit) (1, 13), Love rat unit (19) and Click, Harper and Raper unit (12). Most of these units were generally correlated one another, but only CHR unit had not been directly compared till Stening, Vagne and Grossman have done (11). The present results show that 1 GIH unit is equivalent to 4 units of CHR unit. This result is different from the data of Stening, Vagne and Grossman who estimate the potency of the former is 8 to 9 times greater than the latter. Recently, Konturek reported that CHR unit was roughly 10 times less effective in the stimulation of pancreatic flow and bicarbonate output than GIH unit in conscious cats with cannulated pancreatic ducts (22). As mentioned before in this paper, this discrepancy may be attributed to the difference in the assay method. In April 1970, the third comparison between GIH unit, batch No. 16821, and CHR unit was carried out. At this time also, 1 GIH unit was equal to 4 CHR units. These findings give us the problem in the diagnosis of the pancreatic disorders whether it is reasonable to administer 1 unit of both GIH and CHR unit per kg body weight.

**SUMMARY**

1. The bioassay of secretin using rats was studied. It was clarified that the ligation of the pylorus exerted a serious influence on the pancreatic secretion and on the linear relationship between the responses and the log doses of secretin. The pylorus-ligated rats were more suitable for the assay of secretin than that with normal pylorus.

2. In doses of 0.5 to 8 Crick, Harper and Raper units per rat there was a good linear relationship between the secretin doses and the pancreatic responses.

3. The amount of sodium bicarbonate secreted in the pancreatic juice increased proportionally in doses of 1 to 8 Crick, Harper and Raper units of secretin, but its concentration in response to secretin did not alter.

4. When the potency of unknown preparation was determined with the twin crossover
When Crick, Harper and Raper unit was directly compared with Clinical unit (GIH unit) by this rat method, the potency of the latter was about 4 times greater than that of the former.

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