

## CLINICAL SIGNIFICANCE OF URINARY AMYLASE IN DIAGNOSIS OF PANCREATIC DISEASE

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### ABSTRACT

Studies were made on the methods for urinary amylase determination, stability of the enzyme, isoamylase in urine, normal ranges, diagnostic value in various diseases (pancreatic and non-pancreatic), and relationship between urinary amylase excretion and other laboratory tests. The saccharogenic method of Somogyi was chosen after comparing the three methods of Somogyi, Wohlgemuth, and Van Loon. Somogyi method was modified and simplified to be used for a screening test of pancreatic diseases. Diagnostic values of urinary amylase were evaluated in 54 control subjects, in 73 patients with pancreatic disease, and in 51 with non-pancreatic disease using this simplified method. Urinary amylase excretion was found to be a more sensitive and reliable index than amylase concentration in serum or urine for the diagnosis of acute pancreatitis, also of chronic pancreatitis and of carcinoma. The amylase excretion well reflected the clinical course of pancreatitis and served as a good indicator of its convalescent care. Differential diagnosis may be facilitated by determining urinary amylase excretion in the diseases which have elevated amylase values.

### INTRODUCTION

The determination of serum amylase has been utilized more frequently than any other test to assist the diagnosis of acute pancreatitis. The diagnosis of pancreatitis usually does not present a particular problem in the patients with typical symptoms of acute pancreatitis or elevated serum amylase values. Diagnostic problems are encountered, in fact, in the acute cases that have partially subsided or in the atypical cases with normal or subclinical serum amylase values, and in the mild or atypical cases of chronic pancreatitis or pancreatic carcinoma. In these cases, pancreatic diseases may be overlooked, diagnostic studies may be omitted, or hospitalization may not be indicated. As the result, many cases may be left unrecognized or may be misdiagnosed.

Many workers have demonstrated that the diagnosis of pancreatic disease can be improved with the use of elaborate studies, particularly with Pancreozym-  
Secretin test, roentgenological studies, and isotope scanning. Most of these tests, however, are expensive, time-consuming, and require trained personnel.

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Therefore, the determination of urinary excretion of amylase becomes important in evaluating pancreatic diseases in those cases where the serum amylase values are normal or not significant. Many early investigators believed that urinary amylase was too unreliable to be of any clinical significance. The recent reports of Saxon *et al.*<sup>1)</sup>, Budd *et al.*<sup>2)</sup>, and Gambill *et al.*<sup>3) 4)</sup> indicated that the hourly excretion rate of urinary amylase could be more frequently abnormal in the presence of pancreatic diseases than the serum concentration of either amylase or lipase, and even if both are simultaneously determined. Urinary amylase would be more accurate if given by the amount of amylase per unit of time, because the concentration itself fairly variable.

The purpose of this study is to assess a suitable method for urinary amylase determination among the various methods currently used and to establish the normal range of urinary amylase values. This study also attempted to confirm the clinical significance of the method in the evaluation of patients with various diseases of the pancreas, and to differentiate the pancreatic diseases from the non-pancreatic diseases by the quantitative and qualitative determination of urinary amylase.

#### MATERIALS AND METHODS

##### *I. Case Material*

###### A. Controls:

Control observations were made on 54 healthy persons and patients from the general medical wards who were convalescing from the illnesses without involvement of the pancreas, the liver, the biliary tract, the gastrointestinal tract, and the kidney and with normal renal and hepatic functions. These illnesses were neurosis, hypertension, compensated heart diseases, arthritis, and various pulmonary diseases including bronchitis, asthma, tuberculosis, and emphysema. None of the patients had a previous history suggesting acute or chronic pancreatitis nor had an episode of abdominal complaints during the observation.

###### B. Patients with Pancreatic Diseases:

Seventy-three patients with pancreatic diseases were used for this study (Table 1).

Twenty-four cases of pancreatic carcinoma were all diagnosed at operation or autopsy (two cases), in one of whom amylase excretion was observed at two different stages; first at operation without the presence of liver metastasis and second at autopsy with the presence of liver metastases.

In six the diagnosis of acute pancreatitis was established clinically and in the others at laparotomy. The clinical diagnosis was made on the basis of history, increase of serum amylase and lipase, evidence of one or more complications of acute pancreatitis, roentgenological findings of gastrointestinal

TABLE 1. Patients with Pancreatic Disease

|                       | No. of Patients      |  |       |
|-----------------------|----------------------|--|-------|
|                       | Clinically Diagnosed | Diagnosis verified at Operation or Autopsy | Total |
| Carcinoma of Pancreas |                      |  | 24    |
| Head                  | —                    | 7  |       |
| Body and Tail         | —                    | 9  |       |
| Metastatic Tumor      | —                    | 8  |       |
| Acute Pancreatitis    | 6                    | 2  | 8     |
| Chronic Pancreatitis  | 26*                  | 15**                                       | 41    |
| Total                 |                      |  | 73    |

\* 5 cases of calcified pancreatitis are included.

\*\* 3 cases of calcified pancreatitis are included.

tract, and ruling out of other diseases simulating pancreatitis. The values of urinary amylase were not used for diagnosis. One of the patients was observed in the two stages, with and without renal complication.

Forty-one patients had chronic pancreatitis. The diagnosis was made by one or more of the following criteria:

- (1) gross and/or histologic evidence of chronic inflammation at laparotomy or autopsy (15 cases),
- (2) radiologically demonstrated calcification of the pancreas (8 cases),

TABLE 2. Patients with Non-pancreatic Disease

|   | No. of Patients      |  |       |
|---|----------------------|--|-------|
|   | Clinically Diagnosed | Diagnosis verified at Operation or Autopsy | Total |
| Biliary Tract Diseases                            |                      |  | 23    |
| Cholecystitis                                     | 15                   | 3  |       |
| Idiopathic Choledochus Dilatation                 | —                    | 2  |       |
| Carcinoma   | —                    | 3  |       |
| Liver Diseases                                    |                      |  | 13    |
| Acute Hepatitis                                   | 5                    | —  |       |
| Chronic Hepatitis                                 | 5                    | —  |       |
| Cirrhosis   | 2                    | —  |       |
| Carcinoma   | —                    | 1  |       |
| Peptic Ulcer                                      |                      |  | 12    |
| Stomach   | 2                    | 1  |       |
| Duodenum  | 9                    | —  |       |
| Functional Disturbances of Gastrointestinal Tract | 27                   | —  | 27    |
| Malignancy  |                      |  | 10    |
| Stomach   | 3                    | 2  |       |
| Colon   | 1                    | —  |       |
| Retroperitoneum                                   | 2                    | —  |       |
| Ampulla of Vater                                  | —                    | 2  |       |
| Renal Diseases                                    | 11                   | —  | 11    |
| Diabetes Mellitus                                 | 7                    | —  | 7     |
| Parotitis   | 1                    | —  | 1     |

(3) well-documented previous episodes of acute pancreatitis and recurrence of similar abdominal complaints (8 cases),

(4) abdominal pain and marked disorders of the exocrine function of the pancreas verified by Pancreozymin-Secretin test (13 cases).

The urinary amylase values were not used for these diagnosis.

#### C. Patients with Non-pancreatic Diseases (Table 2):

These patients were diagnosed at operation or autopsy, or by clinical findings including biopsy and endoscopy.

### II. Specimens

In the controls, 2-hour urine specimens before breakfast and after dinner and 24-hour urine specimens were collected on three consecutive days for the determination of normal values of urinary amylase.

In other groups, urine collections were made for 2 hours after dinner in the out-patients, and for 2 hours after dinner and also for 24 hours for consecutive 7 days in the in-patients.

Serum was sampled at least twice during the observation. Patients were placed on no special diet.

### III. Methods

Serum amylase determination was made by the saccharogenic method of Somogyi<sup>5)</sup>. In the controls and in some of the patients with pancreatic diseases, simultaneous determinations were made both by the saccharogenic method of Somogyi and the Wohlgmuth method for comparison<sup>6)</sup>.

The original saccharogenic method of Somogyi and the simplified method developed in this laboratory were used simultaneously for the urinary amylase determination, and a highly significant correlation was found among the values determined by the two methods. After this comparison, urinary amylase determinations were made by the simplified method, except in the presence of albuminuria and glycosuria where the original method and the method of Van Loon, Linkins, and Seger<sup>7)</sup> were used respectively. The values were converted into Somogyi units.

The simplified method in this study differs from the original method in the following points:

The enzyme-substrate mixture incubated at 40°C for 30 minutes is placed in a bath of boiling water to stop the amylase activity. Instead of adding 1.0 ml of 0.3 N Ba(OH)<sub>2</sub> and 1.0 ml of 5 per cent ZnSO<sub>4</sub>·7 H<sub>2</sub>O solution, 2.0 ml of distilled water is admixed with the mixture and cooled in a bath of cold water. A 0.5 ml aliquot of the mixture without centrifugation is pipetted, and the process after this is the same as Somogyi's original method.

For determining the amylase activity after the separation of amylase isozymes, the modified method of Noelting and Bernfeld<sup>8)9)</sup> was used.

## RESULTS

*I. Basic Experiments*

## A. Stability of Urinary Amylase (Table 3):

In eight cases of healthy controls, determinations were made immediately after sampling, after keeping for one week at room temperature, at refrigeration temperature, and at 37°C, respectively. The change of amylase activities of the specimens kept at room temperature and at refrigeration temperature, expressed as the percentage difference from those immediately after sampling, were almost equal (the average difference was +3.9% in the specimens kept at refrigeration temperature and +4.0% in those at room temperature). Only in the specimens kept at 37°C, the decrease of amylase activity was observed (the average difference; -7.6%) but the changes were found to be statistically insignificant among the three groups. Preservation of urine specimens, needed no special attention, but the specimens were preferably kept at refrigeration temperature in the summer. No statistically significant difference was found between the variations of preservation and the technical variations, in which the mean technical errors in the three repeated determinations in each urine specimen from 24 normal controls fell into the average absolute difference of 4.8%.

TABLE 3. Stability of Amylase in Urine

| Urine Specimen   | Initial Value* | Repeated Determinations after One Week |            |         |
|--|----------------|--|------------|---------|
|  |                | Refrigeration Temp.                    | Room Temp. | 37°C    |
| I  | 361            | 363                                    | 351        | 365     |
| II   | 501            | 512                                    | 375        | 336     |
| III  | 186            | 167                                    | 164        | 152     |
| IV   | 229            | 257                                    | 208        | 158     |
| V  | 109            | 106                                    | 104        | 95      |
| VI   | 331            | 332                                    | 354        | 350     |
| VII  | 307            | 363                                    | 337        | 335     |
| VIII   | 344            | 381                                    | 409        | 336     |
| Percentage Difference**                                  |                |  |            |         |
| Mean   |                | 3.88%                                  | 4.00%      | -7.63%  |
| Standard Deviation                                       |                | ±8.54%                                 | ±12.58%    | ±12.65% |
| Absolute Percentage Difference of Technical Variation*** |                |  |            |         |
| Mean   |                | 4.76%                                  |            |         |
| Standard Deviation                                       |                | ±3.77%                                 |            |         |

\* all values are in units per 100 ml.

\*\* difference as percentage of initial value.

\*\*\* absolute difference as percentage of the mean of the three repeated values.

B. Relationship between the Saccharogenic Method of Somogyi and the Simplified Method (Fig. 1 a):

The amylase activity showed a highly significant correlation ( $r=0.95$ ;  $P<0.01$ ) between the simultaneous determinations by the original method of

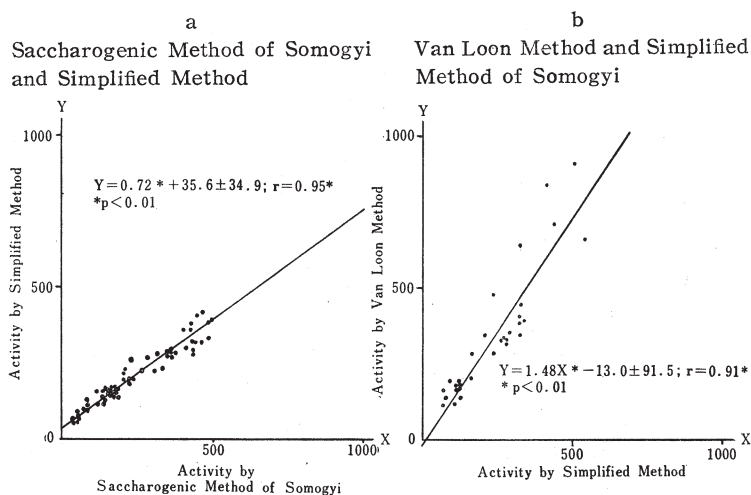


FIG. 1. Relationship of amylase activity detected by different methods.

Somogyi and the simplified method in the same urine specimens from the controls, and the relation was  $y = 0.72x + 35.6 \pm 34.9$  ( $y$ : value by the simplified method,  $x$ : value by the Somogyi method). The simplified method could be used in place of original method of Somogyi except in the presence of albuminuria. In the ranges below 100 Somogyi units (SU), the simplified method gave slightly higher values than the original method, and in the range of normal mean values, 200–400 SU, it gave 85% of the values by the original method. In the range of 500–1000 SU, which is frequently met in pancreatic diseases, the values by the simplified method were about 77% of those by the original method.

C. Relationship between the Method of Van Loon and the Simplified Method (Fig. 1 b):

Amylase determination was made simultaneously by the Van Loon method and the simplified method of Somogyi on 31 urine specimens from healthy persons. A highly significant correlation ( $r = 0.91$ ;  $P < 0.01$ ) was found between the values by the two methods to be expressed by the equation  $y = 1.48x - 13.0 \pm 91.5$  ( $y$ : value by the Van Loon method,  $x$ : value by the simplified method). In the range below 30 units the value by the Van Loon method was lower than by the simplified method, in the range of 200–400 U., the value by the Van Loon method was about 144% of that by the simplified method, and in the range of 500–1000 U., the former was about 146% of the latter.

D. Influence of Urinary Glucose on Amylase Determination (Fig. 2):

The saccharogenic technique has the advantage over iodometric techniques of not being affected by protein constituents which are capable of altering the color reaction between iodine and starch<sup>10,11</sup>). The amylase activity determined

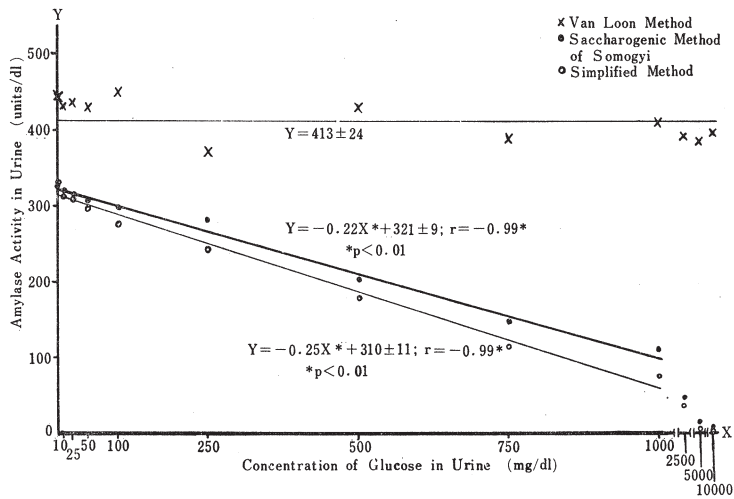


FIG. 2. Influence of glucose on amylase activity in urine.

by the saccharogenic method is liable to be affected by reducing sugar in severe glucosuria. For the comparison of the three methods amylase activity in a normal urine specimen admixed with Dextrose Anhydrous (from Katayama Chemical Co.) was measured simultaneously by the original, the simplified method of Somogyi and by the method of Van Loon. Amylase activity determined by the two saccharogenic method was depressed in direct proportion to the glucose concentration in the urine, and the extent of depression was down to the half at the glucose concentration of about 600 mg/dl and the activity was difficult to measure in the range of glucose concentration above 2000 mg/dl. The relation between amylase activity ( $y$ ) and glucose concentration ( $x$ ) was expressed by  $y = -0.22 x + 321 \pm 9$  ( $r = -0.99$ ;  $P < 0.01$ ) with the original method, and by  $y = -0.25 x + 310 \pm 11$  ( $r = -0.99$ ;  $P < 0.01$ ) with the simplified method. The depression of amylase activity with the Van Loon method was found to be little related to the glucose concentration and not to be significant ( $y = 413 \pm 23$ ), owing to its being an amyloclastic method.

E. Influence of Urinary Albumin on Amylase Determination (Fig. 3):

For the comparison of the influence of urinary protein constituent on three methods, amylase activity of normal urine admixed with human albumin (from Sigma Chemical Co.) was measured simultaneously by the original and the simplified method of Somogyi and by the Van Loon method. Amylase activity ( $y$ ) determined by the original method of Somogyi was little affected by albumin ( $x$ ). The relation was expressed by  $y = -0.03 x + 304 \pm 10$  ( $r = -0.75$ ;  $P < 0.01$ ). With the simplified method the activity was influenced by albumin, but not to the extent of that by glucose, and the relation was  $y = -0.17 x + 341$

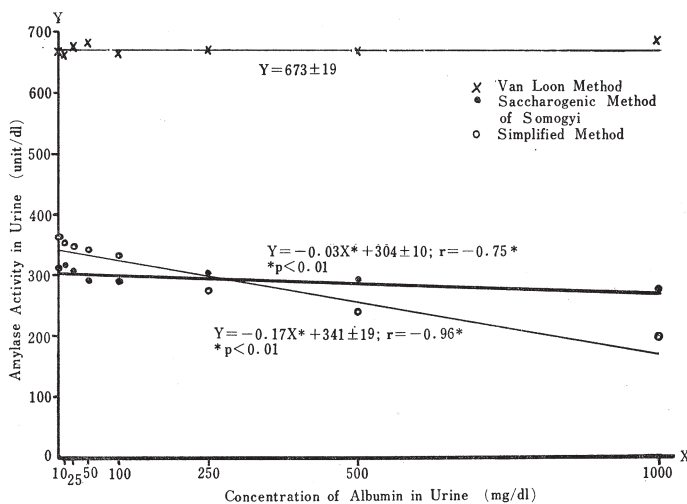


FIG. 3. Influence of albumin on amylase activity of urine.

$\pm 19$  ( $r = -0.96$ ;  $P < 0.01$ ). With the method of Van Loon, albumin did not influence the amylase activity so significantly as described by the workers<sup>10,11</sup> of isoamylase who studied with the iodo-metric methods. This might be due to the amount of urine specimen used in the determination which was as small as 0.1 ml diluted 20 times.

In the determination with the method of Van Loon, the optimum range of amylase activity is limited and the color reaction between iodine and starch rapidly alters, so that the technical variations are likely influence the result easily, even though the activity per se is not so much influenced by glucose and albumin in the urine. In order to use the urinary amylase activity in the screening test of pancreatic diseases, a method should be devised which allows testing of amylase activities in a large number of urine specimens at one time with stable color reaction and little technical variation. The simplified saccharogenic method of Somogyi developed in this laboratory serves for the purpose better than the method of Van Loon for the urine specimens without albumin or glucose, though the process of the former is more complicated than that of the latter.

## II. Clinical Observation

### A. Observation of the Controls

#### a) Urinary Amylase Activity Determined by Wohlgemuth Method:

In order to reevaluate the diagnostic significance of the Wohlgemuth method and the normal range of amylase with this widely used method, amylase determinations were carried out on 458 urine specimens from the 54 controls. The values distributed below 64 Wohlgemuth units (WU); 2 WU in 6.8%, and



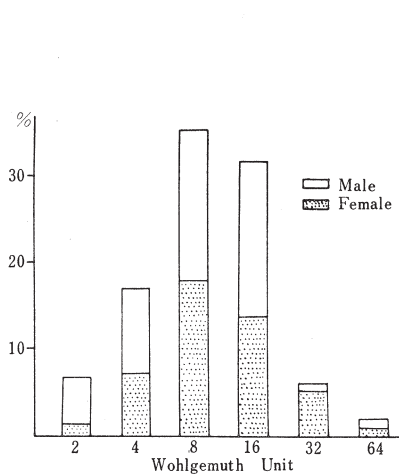


FIG. 4. Distribution of wohlgemuth values in 458 urine specimens from 54 healthy subjects.

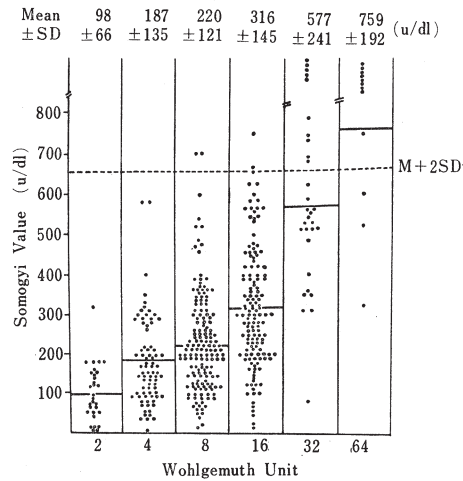


FIG. 5. Relationship between Wohlgemuth value and Somogyi value (u/dl) of amylase concentration in urine.

64 WU in 17.3%, 8 WU in 35.6%, 16 WU in 31.8%, 32 WU in 6.1%, and 64 WU in 2.6% of these determinations (Fig. 4). The normal range of Wohlgemuth values has been assumed to be between 16 and 32 WU, but the values in the determinations were below 64 WU and a half of them were below 8 WU which have been considered as below-normal values<sup>12)</sup>. The values between 2 and 8 WU are virtually impracticable to interpret as abnormal.

The relationship between the Wohlgemuth and the Somogyi values was studied on 456 urine specimens of the controls and of some patients with pancreatic diseases by simultaneous determinations with the two methods (Fig. 5). As a whole, it was demonstrated that there was a statistically significant correlation ( $P < 0.01$ ) between the Wohlgemuth unit and the Somogyi amylase concentration; Somogyi values increased proportionally with the increase of Wohlgemuth units. However, the Somogyi concentrations of the specimens giving the same Wohlgemuth unit varied and scattered fairly widely, so that there were no statistically significant difference between the two means of Somogyi values each corresponding to the two neighbouring Wohlgemuth units, *i.e.* in 2 WU vs. 4 WU vs. 8 WU, while some significant differences were demonstrated between the means of Somogyi values corresponding to 8 WU vs. 16 WU ( $P < 0.01$ ), 16 WU vs. 32 WU, and 32 WU vs. 64 WU ( $P < 0.05$ ). The overlaps of the Somogyi values corresponding to the two neighbouring Wohlgemuth units due to the wide scattering could be explained by that the method of Wohlgemuth is semi-quantitative and the color reaction between iodine and starch is inhibited by some constituents in the urine. There were observed above-normal values (normal mean plus 2 standard deviations) of Somogyi

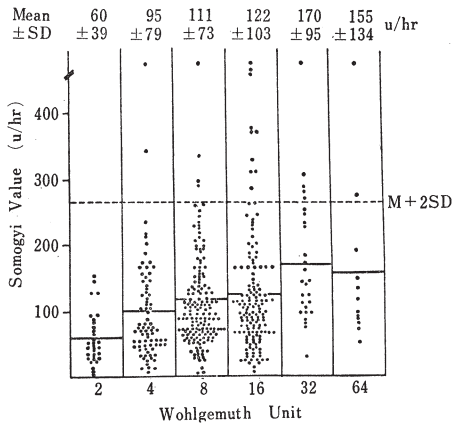


FIG. 6. Relationship between Wohlgemuth value and Somogyi value (u/h) of amylase excretion in urine.

concentration in more than half a number of the specimens giving 64 WU. The Wohlgemuth values from 64 to 128 WU have been regarded as the normal upper limit, but in the cases of oliguria the specimens giving 64 WU to 128 WU often corresponded to the normal values of excretion determined by the Somogyi method. On the other hand, some of the urine specimens giving 8 to 32 WU corresponded to above-normal values of amylase concentration measured by the Somogyi method.

The relationship between the Wohlgemuth units and the Somogyi values of amylase excretion per hour is shown in Fig. 6. Both the Wohlgemuth values and the amylase excretions increased with a significant correlation ( $P < 0.01$ ). However, no significant differences were demonstrated between the two means of amylase excretions per hour corresponding to the two neighbouring Wohlgemuth units, except between those corresponding to 16 WU vs. 32 WU ( $P < 0.05$ ). Above-normal values of amylase excretion per hour were observed in the specimens of which Wohlgemuth units covered widely from 4 WU to 64 WU.

The relationship between the Wohlgemuth units and the urinary volume was an inverse correlation ( $P < 0.01$ ) as shown in Fig. 7, but there were no

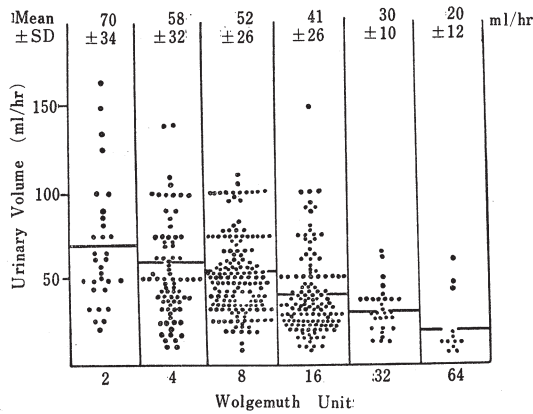


FIG. 7. Relationship between Wohlgemuth value and urine volume (ml/h).

significant differences between the two means of urinary volumes of the specimens corresponding to the two neighbouring Wohlgemuth units except between those corresponding to 8 WU vs. 16 WU ( $P < 0.01$ ).

b) Urinary Amylase Determined by Somogyi Method:

The amylase concentration determined by the Somogyi method was inversely related to the urinary volume ( $r = -0.42$ ;  $P < 0.01$ ) as shown in Fig. 8 a, and the amylase excretion per hour was not so directly related to the urinary volume ( $r = +0.26$ ;  $P < 0.01$ ) as shown in Fig. 8 b, but the amylase concentration and excretion showed a considerable correlation ( $r = +0.76$ ;  $P < 0.01$ ). Above-normal values of amylase excretion were observed in some urine specimens of large volume, even when the concentration were low.

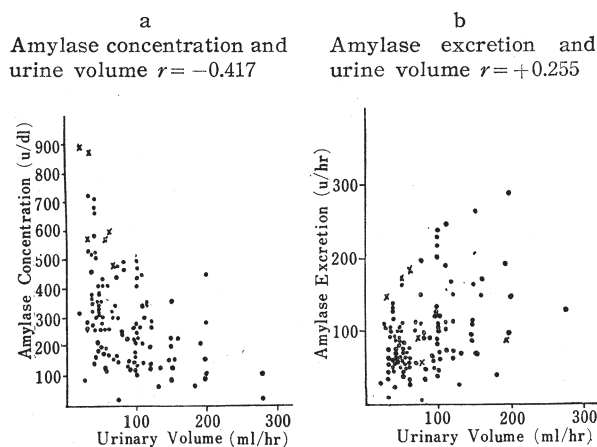


FIG. 8. Relationship between urine volume and Somogyi values of amylase concentration and excretion in urine.

c) Normal Values:

Table 4 shows the normal values of amylase concentration and excretion determined by the Somogyi method on 2-hour urine specimens before breakfast and after dinner, and on 24-hour specimens obtained from 54 controls for three consecutive days. The average amylase concentration was the highest in the specimens after dinner (346 u/dl), and the average excretion was the highest in the specimens before breakfast (117 u/h), but there was no significant difference among the mean values of the amylase concentrations and of the excretions observed in the three urine collections. The differences between male and female were also proved to be insignificant. The mean plus 2 standard deviations of concentration and excretion were chosen respectively to use as the upper limit of normal urinary values, namely, 650 u/dl for the concentration, and 249 u/h or 5016 u/day for the excretion. However, the definite lower limit of the values could not be established in this study.

TABLE 4. Normal Values of Urinary Amylase

|                          | 2-h. before<br>Breakfast |          | 2-h. after Dinner |          | 24-h. Period |          |           |
|--------------------------|--------------------------|----------|-------------------|----------|--------------|----------|-----------|
|                          | Conc.*                   | Output** | Conc.*            | Output** | Conc.*       | Output** | Output*** |
| Mean Value               | 315                      | 117      | 346               | 109      | 258          | 105      | 2520      |
| Standard Deviation       | 147                      | 75       | 152               | 70       | 139          | 52       | 1248      |
| Upper Limit (M+2SD)      | 609                      | 297      | 650##             | 249#     | 536          | 209      | 5016###   |
| Coefficient of Variation | 0.47                     | 0.64     | 0.44              | 0.64     | 0.54         | 0.50     | 0.50      |

\* Somogyi Unit/100 ml

\*\* Somogyi Unit/h

\*\*\* Somogyi Unit/24 h

# used as the upper limit of the hourly output after meal.

## used as the upper limit of the concentration.

### used as the upper limit of the daily output.

#### B. Urinary Amylase in Patients with Pancreatic Disease (Table 5):

The cases exhibiting serum and urinary amylase concentration and urinary excretion of above-normal values more than once in a week consisted 28.8%, 27.4% and 56.2% of the 73 cases studied, respectively. The ratio of abnormal amylase excretion was significantly higher than the ratios of abnormal serum amylase concentration and urinary amylase concentration ( $P < 0.001$ ). The case ratios of above-normal values of urinary amylase concentration and excretion were 23.7% and 49.4% of the 59 cases in which urine specimens were collected for 2 hours after dinner, and were 27.4% and 56.2% of the 73 cases of 24-hour urine collection, respectively. The case ratio of abnormal values in amylase excretion was significantly higher than that in amylase concentration in both methods of urine collection, but there was no significant difference in the abnormality ratio of the concentration and the excretion between the two different methods of urine collection.

##### a) Carcinoma of the Pancreas:

Five of the 7 patients with carcinoma of the head of the pancreas, 3 of the 9 patients with carcinoma of the body and tail, and 6 of the 8 patients with metastatic carcinoma of the pancreas showed amylase excretion exceeding the normal range at least once a week. Elevation of urinary amylase excretion was rather continuous in carcinoma of the pancreas, and the values above normal were observed at least twice a week except in one case in each of the three groups of carcinoma.

Carcinoma with liver metastasis was diagnosed at laparotomy or autopsy in 3 of the 7 patients with carcinoma of the head of the pancreas, and in 7 of the 9 patients with carcinoma of the body. Values of amylase excretion above normal were observed in 3 of the 10 cases with liver metastasis and in all of the 6 cases without liver metastasis, and the difference in the frequency of urinary amylase abnormalities was significant ( $P < 0.05$ ). On the other hand,

TABLE 5. Urinary Amylase in Pancreatic Disease

| Disease  | Range of Values           | 24-hour Urine Collection           |   | 2-hour Urine Collection after Meal |   |
|--|---------------------------|------------------------------------|---|------------------------------------|---|
|  |                           | Conc. (u/dl)                       | Excretion (u/d)   | Conc. (u/dl)                       | Excretion (u/day)   |
| Carcinoma of Head of Pancreas                          | M-SD><br><M+2SD<br><M+3SD | 1, 1, 1, 7/7<br>1, 7/7             | 1, 3, 5, 7, 7/7<br>1, 2, 5, 7/7                           | 6, 7, 7/6<br>2, 5, 6/6             | 3, 7/6<br>1, 5, 7, 7/6<br>3, 7, 7/6   |
| Carcinoma of Body and Tail of Pancreas                 | M-SD><br>>M+2SD<br>>M+3SD | 1, 2/9<br>1/9                      | 2, 3, 5, 6, 7, 7, 7/9<br>1, 5, 7/9<br>1, 2, 2/9           | — 2/8<br>0/8                       | 2, 7/8<br>2, 4, 7/8<br>2, 3/8   |
| Metastatic Tumor of Pancreas                           | M-SD><br>>M+2SD<br>>M+3SD | 2, 7, 7/8<br>1, 7, 7/8             | 1, 4, 6, 6, 7, 7/8<br>1, 4, 6/8                           | — 4/3<br>3/3                       | 2/3<br>1/3<br>1/3   |
| Acute Pancreatitis                                     | M-SD><br>>M+2SD<br>>M+3SD | 1, 1, 2, 3, 3/8<br>1, 1, 1, 1, 1/8 | 2, 2, 4, 5, 5, 6, 6, 7/8<br>1, 1, 2, 3, 4/8               | — 5/2<br>1, 5/2                    | 0/2<br>4, 7/2<br>1, 6/2   |
| Chronic Pancreatitis Diagnosed at Operation or Autopsy | M-SD><br>>M+2SD<br>>M+3SD | 2, 4, 5/15<br>1, 1, 1/15           | 1, 2, 3, 5, 7, 7/15<br>1, 2, 2, 2, 5, 7/15<br>5, 7/15     | — 3/12<br>1, 1, 1/12               | 1, 2, 2, 2/12<br>1, 5, 7, 7/12<br>1, 3, 4, 7/12                               |
| Chronic Pancreatitis with Calcification                | M-SD<<br>>M+2SD<br>>M+3SD | — 5/7<br>4/7                       | 1, 5, 5, 7/7<br>3, 7/7<br>7/7                             | — 3/6<br>1/6                       | 1, 4/6<br>1, 7/6<br>7/6   |
| Chronic Pancreatitis Diagnosed Clinically              | M-SD><br>>M+2SD<br>>M+3SD | 2, 3/18<br>3/18                    | 1, 1, 1, 2, 3, 3, 3, 4, 5, 5, 7/18<br>1, 1, 2, 2, 3, 5/18 | — 4/22<br>2, 3, 3/22               | 0/22<br>1, 1, 1, 2, 2, 2, 3, 3, 6, 7, 7, 7, 7/22<br>1, 1, 1, 3, 4, 4, 5, 7/22 |

Note: Each figure of numerators shows the number of occurrence within a week. The denominator shows the number of cases studied.

subnormal values (below the mean minus a standard deviation) were detected in 9 of the 10 cases with liver metastasis, but in none of the 6 cases without liver metastasis. The frequency of the subnormal values was significantly higher in patients with liver metastasis than in those without ( $P < 0.05$ ).

Case 1. Carcinoma of the Body of the Pancreas (Fig. 9)—A 45 years-old housewife was admitted to the hospital on May 21, 1967, because of dull epigastric and back pain. Pancreozymin-Secretin test revealed a slightly lowered output of amylase, but normal bicarbonate concentration and normal volume of duodenal aspirate. Urinary amylase excretion was abnormally elevated before the operation. Surgical exploration revealed carcinoma of the body of the pancreas without liver metastasis. The tumor measured  $4 \times 5 \times 6$  cm. Urinary amylase excretion was abnormally elevated for about 10 days after the laparotomy, and then gradually decreased and became below 1000 u/day a week before her death. Serum amylase remained normal throughout the hospital course. She died on September 28, and at autopsy the pancreas was involved in a mass of carcinoma with metastases to the liver.

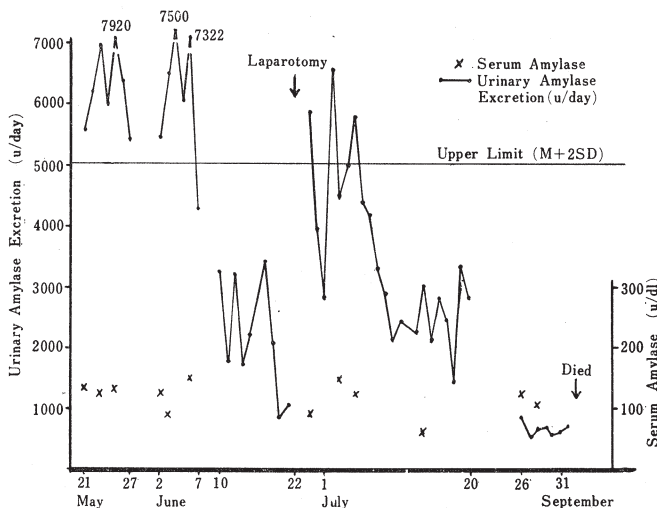


FIG. 9. Case 1. Carcinoma of body of pancreas  
F. Y. Age 45, Housewife.

#### b) Pancreatitis:

Acute Pancreatitis: Serum and urinary amylase values above normal were observed in all the cases of acute pancreatitis. The urinary amylase excretion exhibited a greater degree of abnormality than the corresponding serum levels, and whenever the serum concentration was abnormal, the urinary amylase was in the abnormal range. After the serum amylase rapidly returned to the normal range, the urinary amylase yet remained abnormally increased for a

longer period (1 to 60 days).

Case 2. Acute Pancreatitis (Fig. 10)—A 32 years old man was hospitalized three days after the onset of severe upper abdominal pain and shock. Serum amylase value on admission was 412 u/dl, but returned to the nondiagnostic range on the following day. Urinary amylase excretion returned to the normal range in a week, but it again began to increase abnormally after the feeding on the 13th day. A greater degree of abnormality of amylase excretion was observed in 2-hour urine collection after dinner than in 24-hour collection, and the 2-hour urinary amylase excretion became abnormal with the increase of diet and exercise for a longer period. On the 65th hospital day he was operated on for gallstone and postinflammatory abscess in the retroperitoneum. Cholecystectomy, sphincterotomy, and external drainage from the abscess cavity performed. Abnormal values of urinary amylase excretion were found occasionally even 3 months after the operation.

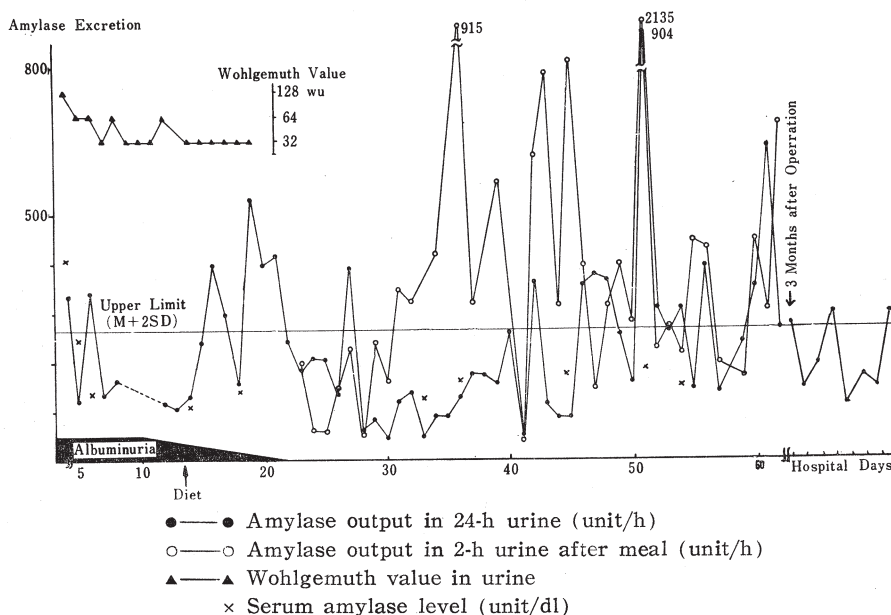


FIG. 10. Case 2. Acute Pancreatitis M. U. Age 32, Male.

Chronic Pancreatitis: In 10 of the 41 patients with chronic pancreatitis and in 19 of them, high abnormal values of serum amylase concentration and urinary amylase excretion were obtained respectively. The amylase excretion exceeding the upper limit of the normal was observed in 5 of the 15 surgically detected cases, in 2 of the 7 cases of pancreatic calcification, and in 12 of the 18 cases diagnosed clinically. Incidences of cases of above-normal excretion

were lower in the groups of surgical detection and pancreatic calcification than in the group of clinical diagnosis. The duration of abnormal amylase excretion was shorter in chronic pancreatitis than in other pancreatic diseases. The frequencies of cases of the subnormal values of amylase excretion below the mean value minus a standard deviation were higher in the group of surgical detection and calcification, but were not significantly higher than in healthy controls.

Case 3. Chronic Pancreatitis with Calcification (Fig. 11)—A 46 years old man was admitted to the hospital on September 18, 1965, because of upper abdominal pain and ascites. Abnormally high values of amylase were demonstrated in serum, urine, and ascites. Pancreatic calcification was shown radiologically, and at laparotomy on June 17, 1966, enlarged, thickened, inflamed pancreas was detected but with no mass of carcinoma. Sphincterotomy was performed with diagnosis of chronic calcified pancreatitis, and after that he remained free of symptom and his urinary amylase excretion continued to be lower than 1,000 u/day.

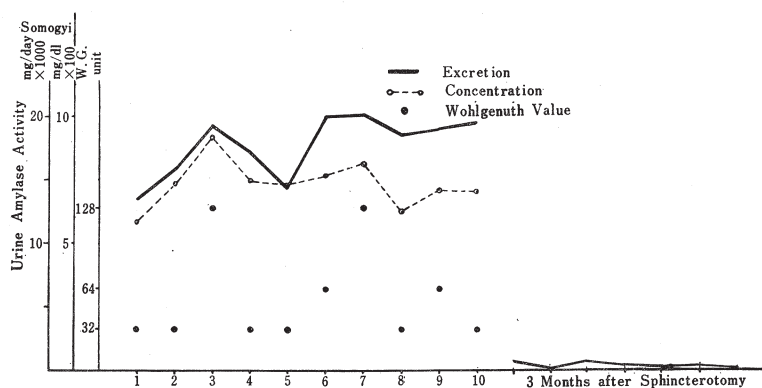


FIG. 11. Case 3. Chronic pancreatitis  
M. A. Age 46, Male.

Case 4. Chronic Relapsing Pancreatitis (Fig. 12)—A 28 years old man who had a previous history of acute pancreatitis revealed at surgical exploration was referred to the hospital on the 25th day of his acute exacerbation of pancreatitis. Urinary amylase excretion gradually returned to normal with intermittent above-normal values and well reflected the clinical course of the patient. On the other hand, serum amylase had been already reduced to normal before his recovery, and urinary amylase concentrations determined by both the methods of Somogyi and Wohlgenuth were non-diagnostic because of being affected by urinary volumes and failed to reflect the clinical course.



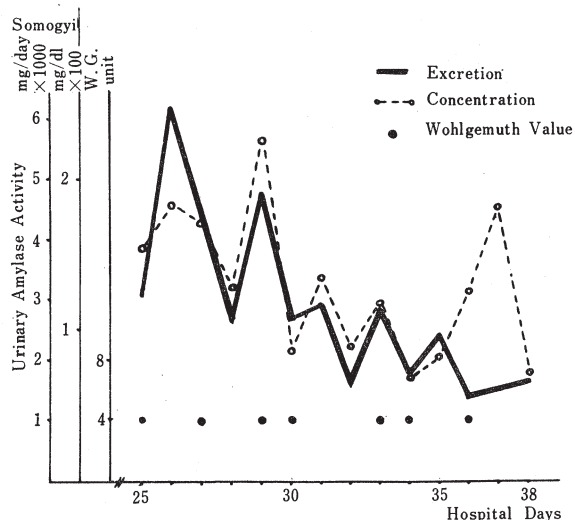


FIG. 12. Case 4. Chronic Pancreatitis  
T. T. Age 28, Male.

### C. Urinary Amylase in Patients with Non-pancreatic Disease (Table 6):

Urinary amylase excretions were abnormally high in 3 of the 19 patients with biliary tract diseases (cholecystitis 2; cancer 1), but serum amylase levels were all non-diagnostic. Subnormal values (below the mean values minus a standard deviation) were detected in 7 of the 19 cases.

In the 14 patients with liver disease, above-normal urinary excretions were observed in 4 of the 5 patients with acute hepatitis, 1 of the 5 with chronic hepatitis, and in 1 of the 3 with liver cirrhosis, but serum amylase values were non-diagnostic in all the cases. Subnormal values (below the mean values minus a standard deviation) were detected in 4 of the 14 cases.

Above-normal urinary amylase excretions were detected in 3 of the 11 cases with peptic ulcer and in 1 of the 27 patients with functional disorders of the intestine, but serum amylase values were all normal in the two groups.

Above-normal values of urinary amylase excretion and serum amylase were detected in 3 (Carcinoma of Papilla Vateri 2; Retroperitoneal Tumor 1), and in 1 patient (Carcinoma of P. Vat.) of the 10 patients with malignant tumors, respectively.

In the 7 patients with diabetes mellitus, above-normal and subnormal values for urinary amylase excretion were detected in 1 and in 3 patients, respectively.

In the 11 patients with renal disease no abnormally high value was detected and in 10 of the 11 patients only subnormal values of urinary amylase excretion were detected throughout the observation. In the 2 cases of azotemia abnormally high levels of serum amylase were detected.

TABLE 6. Urinary Amylase in Non-pancreatic Diseases

| Disease                               | Range of Values           | 24-hour Urine Collection |   | 2-hour Urine Collection after Meal |  |
|---------------------------------------|---------------------------|--------------------------|---|------------------------------------|--|
|                                       |                           | Conc. (u/dl)             | Excretion (u/day)   | Conc. (u/dl)                       | Excretion (u/h)  |
| Biliary Tract Diseases                | M-SD><br>>M+2SD<br>>M+3SD | 2*/19<br>2*/19           | 1, 1, 1, 3, 4, 4, 7*/19<br>1, 1/19<br>1/19  | 1*/22<br>1*/22                     | 1, 1, 1, 2, 4*, 5*/22<br>1*, 2/22<br>1, 1*/22  |
| Liver Diseases                        | M-SD><br>>M+2SD<br>>M+3SD | 0/14<br>0/14             | 2 <sup>ca</sup> , 3 <sup>ca</sup> , 5 <sup>ci</sup> , 7 <sup>ca</sup> /14<br>1 <sup>a</sup> , 1 <sup>a</sup> , 1 <sup>ch</sup> , 1 <sup>ci</sup> , 2 <sup>a</sup> /14<br>0/14 | 0/10<br>0/10                       | 1 <sup>ca</sup> /10<br>1 <sup>ci</sup> , 1 <sup>ch</sup> /10<br>0/10                     |
| Peptic Ulcer                          | M-SD><br>>M+2SD<br>>M+3SD | 0/7<br>0/7               | 1 <sup>d</sup> , 7 <sup>d</sup> /7<br>1 <sup>d</sup> , 1 <sup>g</sup> , 6 <sup>d</sup> /7<br>4 <sup>d</sup> /7  | 0/9<br>0/9                         | 3 <sup>d</sup> , 5 <sup>d</sup> /9<br>0/9<br>0/9   |
| Functional Disturbances of G.I. Tract | M-SD><br>>M+2SD<br>>M+3SD | 0/11<br>0/11             | 1, 2, 3/11<br>1/11<br>0/11  | 0/21<br>0/21                       | 1, 1/21<br>0/21<br>0/21  |
| Malignant Tumors                      | M-SD><br>>M+2SD<br>>M+3SD | 0/11<br>0/11             | 1, 3, 5, 6, 7, 7/11<br>1 <sup>a</sup> , 1 <sup>p</sup> /11<br>1 <sup>p</sup> /11  | 1 <sup>p</sup> /5<br>0/5           | 4, 5/5<br>3, 4 <sup>p</sup> , 6 <sup>p</sup> /5<br>1, 1 <sup>p</sup> , 2 <sup>p</sup> /5 |
| Diabetes Mellitus                     | M-SD><br>>M+2SD<br>>M+3SD | 0/7<br>0/7               | 2, 6, 7/7<br>5/7<br>0/7   | 0/3<br>0/3                         | 2, 3/3<br>0/3<br>0/3   |
| Renal Diseases                        | M-SD><br>M><br>>M+2SD     | 0/11                     | 1, 1, 2, 2, 3, 5, 6, 6, 7/11<br>1, 5, 7, 7, 7, 7, 7, 7/11<br>0/11   | —<br>—<br>0/5                      | 3, 4, 4/5<br>1, 3, 5, 7, 7/5<br>0/5  |

Note: \*): Choledochus carcinoma, ca): Carcinoma of the liver, ci): Liver cirrhosis,

a): Acute hepatitis, ch): Chronic hepatitis, p): Carcinoma of the papilla Vateri.

Each figure of numerators shows the number of occurrence within a week.

The denominator shows the number of cases studied.

In a patient with parotitis, values above the normal were detected both in serum and urine.

In only 4 cases of non-pancreatic diseases above-normal serum amylase levels (Renal Insufficiency 2; Carcinoma of Papilla Vateri 1; Parotitis 1) were detected. In almost all the cases with abnormally high excretion above-normal values of urinary amylase excretion occurred only once a week and the frequency of subnormal values was not more significant than that of controls except in renal diseases.

D. Comparison between Urinary Amylase Excretion and the Data of Other Tests:

a) Pancreozymin-Secretin Test:

The duodenal aspirates were collected by Bartelheimer's tube with double balloons for 10 minutes after Pancreozymin stimulation (1 unit per kg body weight by intravenous route) and for (10, 10, 20, 20) minutes after Secretin stimulation (same as Pancreozymin). The volume and the content of amylase and bicarbonate were measured in each collection.

Above-normal values of urinary amylase were observed in 24 of the 40 patients with impaired pancreatic secretion, and in 8 of the 30 patients with normal pancreatic secretion. The incidence of urinary amylase abnormalities was significantly higher in the former group than in the latter ( $P < 0.001$ ). There was no significant correlation between urinary amylase and any of the three factors of the aspirate (amylase output, maximum bicarbonate concentration, and volume). Subnormal values of urinary amylase were demonstrated in 10 of the 40 patients with the impaired secretion, and in 4 of the 30 with the normal secretion. The frequency of subnormal amylase excretion in the former group was slightly higher than that in the latter but not significantly.

b) Provocative Serum Enzyme Test by neostigmine (Vagostigmin Test):

Above-normal values of urinary amylase were found in 4 of the 12 patients with abnormal results of the test, and in 13 of the 41 with normal results. There was no significant difference in urinary amylase excretion between the former and the latter groups.

c) Glucose Tolerance Test:

In 6 of the 9 patients with diabetic curve in the test, and in 6 of the 30 with normal glucose tolerance curve, urinary amylase excretions exceeded the upper limit of normal. Urinary amylase excretions were elevated more frequently in the patients with diabetic curves than in those with normal curves with the statistical significance of  $P < 0.05$ .

d) Serum Bilirubin Level:

Urinary amylase values were elevated in 7 of the 8 jaundiced patients, and in 10 of the 18 non-icteric patients. There was a tendency to elevation of urinary amylase in the jaundiced cases but not significantly.

e) Serum Alkaline Phosphatase:

Urinary amylase values were abnormally high in 7 of the 11 cases with elevated alkaline phosphatase, and in 8 of the 14 with normal alkaline phosphatase. No significant difference of urinary amylase was found between the two groups.

f) Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT):

Amylase excretions were above the normal in 9 of the 16 cases with elevated SGOT and in 26 of the 42 cases with normal SGOT, and in 9 of the 13 cases with elevated SGPT and in 27 of the 45 with normal SGPT. There was no significant correlation between urinary amylase and the two transaminase values.

g) Serum Lactic Dehydrogenase (LDH):

In the patients with pancreatic carcinoma, 6 of the 7 cases with high LDH levels and 6 of the 14 cases with normal LDH levels exhibited above-normal amylase excretion, and the frequency of urinary amylase abnormalities in the former was significantly higher than in the latter. ( $P < 0.05$ ).

E. Isoenzymes of Urinary Amylase (Fig. 13):

Amylase activities in the urine were separated by means of the three fractionating methods: paper electrophoresis, polyacrylamide gel electrophoresis, and Sephadex G-100 gel filtration<sup>13) 14) 15) 16) 17) 50)</sup>. Amylase activities were estimated by a modification of the saccharogenic method of Noelting and Bernfeld<sup>8) 9)</sup>.

The saccharogenic activity in both normal and "pancreatitis" urine was largely confined to the  $\gamma$ -globulin zone, with only trace amount of activity of questionable significance in the other electrophoretic areas of both paper and polyacrylamide gel electrophoresis. The activity fractionated by Sephadex G-100 gel filtration was found also largely in the main peak and as a trace in other questionable peaks. The elevation in amylase activity that occurred in pancreatitis was consistently confined to the area in which amylase has been assumed to be of pancreatic origin.

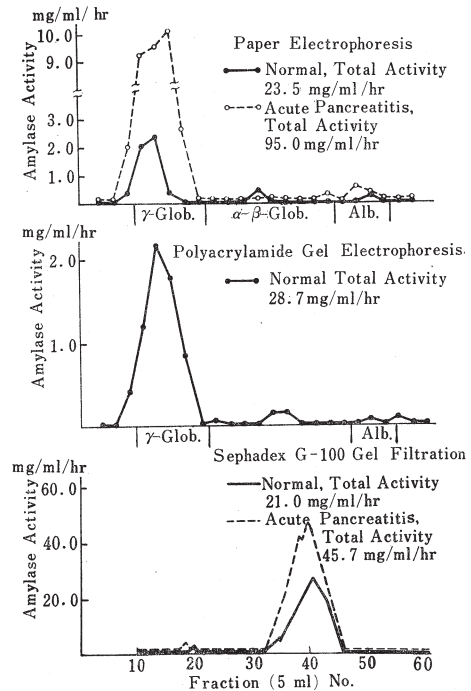


FIG. 13. Pattern of urinary amylase isozyme in normal and acute pancreatitis.

## DISCUSSION

**Stability of Amylase Activity:** The activity of amylase in the urine allowed to stand at refrigeration temperature, room temperature, or at 37°C, for one week showed no statistical difference. Stocks<sup>18)</sup>, Saxon<sup>1)</sup>, and Budd<sup>2)</sup> observed a similar stability of amylase in the urine tested by allowing the specimens to stand at room or refrigeration temperatures for as long as one week. Hayashi and his colleagues<sup>19)</sup> showed that exposure of the serum specimen pooled from the patients with acute pancreatitis to 45°C for 16 hours caused a marked reduction in amylase activity but this storage temperature had little effect on the saccharogenic activity of the normal pool. In the present study a marked reduction in the amylase activity was not observed in the urine specimens from the patients with pancreatic diseases. However, amylase determination should be done in the urine specimens immediately after collection or after being stored in a refrigerator until determination.

**Methods for Amylase Determination:** Two principal techniques are generally employed to measure the amylase activity in serum and urine: the amyloclastic method and the saccharogenic method. Most of the amyloclastic methods are simpler in the process than the saccharogenic techniques and have been generally used in many laboratories. Most of the studies on urinary amylase have utilized the amyloclastic methods. The color reaction between iodine and starch in the amyloclastic technique is inhibited by protein constituents<sup>10)</sup> (esp. albumin) and the usual contents of urine (uric acid, urea, etc.)<sup>20)</sup>. Although amylase activities in the urine specimens admixed with human albumin in this study were not affected by human albumin at different concentrations, the starch-iodine color reaction is readily altered in the course of time, and this is one of the causes of the wide variation in the activities. An amyloclastic method should be used only for the amylase determination in the presence of glycosuria and only by a well-trained technician. Amylase determinations by the saccharogenic method of Somogyi show little variations except in the presence of glycosuria and are hardly influenced by urinary constituents, such as proteins, urate, urea, and so on. Dozzi also demonstrated the superiority of the saccharogenic method of Somogyi over the other techniques for determining urinary amylase<sup>21)</sup>. However, the saccharogenic method of Somogyi is complicated, and is troublesome for the amylase determination of the urine free of proteins. The simplified saccharogenic method of Somogyi used in this study requires only about half of the time needed for the original method and has a highly significant correlation to the original when they are compared at the simultaneous determination of amylase activity in the urine specimens containing little glucose. The superiority of the simplified method over the other methods are in the stability of the color reaction and in the simplicity of the techniques when a large number of urine specimens are determined at

one time for amylase activity. The amylase determination in the simplified method of Somogyi could be done in a simpler and more accurate way by the following procedures: After adding 0.5 ml of urine to test tubes, 2.5 ml of the starch solution are added into each of the tubes by a Auto-injector set to add 2.5 ml of the fluid accurately and serially. This makes it possible to start the enzyme-substrate reaction in a great number of tubes at the same time, and the reaction could be also stopped by placing the tubes in a large boiling water bath after 30 minutes incubation. The time of the reaction between amylase and starch is made constant even in a large number of the determinations. The color reaction is stable and does not alter at least within a few hours or so throughout the colorimetry is done. If the urinary amylase determination is done as above, it is easy to use on many patients as a screening test for the diagnosis of pancreatic diseases.

The Wohlgemuth method has been widely used because of its simplicity in the technique, but amylase values determined by this method are not accurate because of its semiquantitative procedures and the influence of other urine substances on the color reaction<sup>20)</sup>. This method also does not yield reliable results<sup>15)22)</sup> particularly in the bordering range of normal values (32-128 WU), as suggested by Fried, *et al.*<sup>23)</sup> The Wohlgemuth method has its diagnostic disadvantages since it uses urinary amylase concentration for detecting abnormalities, not the amount per unit of time. Thus, the frequency of abnormality by the Wohlgemuth method was one-sevenths of the abnormality ratio determined by urinary amylase excretion in the patients with pancreatitis<sup>15)</sup>.

Normal Values: The normal range of Wohlgemuth values has been assumed to be between 16 and 32 units, and the values from 2 to 8 units have been considered abnormally low and as a sign of pancreatic exhaustion<sup>12)</sup>. The values from 2 to 8 units have been obtained in this study and are difficult to be interpreted as abnormal. The decrease of urinary amylase excretion is possible after pancreatic exhaustion, but such a pathological significance could not be confirmed by only such Wohlgemuth values as 2 to 8 units. Patients with pancreatitis often show above-normal values for urinary amylase excretion with adequate volume of urine, though they may give the Wohlgemuth values as low as 4 to 8 units throughout the observation. When compared with Somogyi units, the normal upper limit of the Wohlgemuth values is considered to be at 64 units, and the normal range of Wohlgemuth values should be between 2 and 64 units.

In this study, the upper limit of normal urinary amylase was chosen as the mean value plus 2 standard deviations, 5016 units/day or 249 units/h. The mean value of 2,520 units/day with a standard deviation of 1,248 as the 24-hour urinary amylase excretion in the present paper corresponds well with the results of many investigators:  $3,095 \pm 1,024$  u/day by Smith and Roe<sup>24)</sup>,  $1,947 \pm 662$  u/day by Saxon<sup>1)</sup>,  $2,926 \pm 1,074$  u/day by Budd<sup>2)</sup>,  $148 \pm 61$  u/h by Kirschen<sup>25)</sup>,

and  $119 \pm 53$  u/h by Calkins<sup>26</sup>. The mean values of urinary amylase excretion before breakfast, after dinner, and during 24 hours were compared to evaluate the diurnal variations and the effect of meals on the excretion, but no significant difference was found among them. Similar results were obtained by Saxon<sup>1</sup> and Sung<sup>27</sup>. The variations were observed in urinary amylase excretion after dietary changes by Smith and Roe<sup>24</sup>, and the relationship between age and serum amylase was studied by Ahlert and his colleagues<sup>28</sup>. No special relationship was found in diets or ages, but a slightly wide standard deviation in this study might have been found because no particular limitation to diets or ages in the controls was made.

The lower limit of normal values could not be established in the present paper, as many investigators failed to set a definite lower limit. Low values are frequently observed at the late stages of pancreatic diseases and these have been assumed to be related to pancreatic exhaustion. These low values may indicate progressive destruction of the gland, but the significance of these values could be determined only by histological examinations of the entire pancreas after total pancreatectomy or at autopsy within a few days after the low urinary amylase values are obtained—these circumstances do not occur in sufficient numbers to allow a satisfactory evaluation. In addition, other debilitating conditions have been reported to give low values of amylase excretion<sup>29</sup>.

Urinary amylase excretion remains rather constant over a wide range of urinary flow, but in this study, not to such extent as observed by McGeachin<sup>30,31</sup>. Patients with daily urinary volume of below 500 ml often gave lower values for amylase excretion. Amylase concentration was inversely correlated with urinary volume, as studied by McGeachin<sup>30,31</sup>. This might have caused many early investigators to believe that the urinary amylase was too unreliable to be of any clinical significance and was inferior to the serum determination.

Since meals may initiate a provocative effect on the pancreas and clinical exacerbation has often been observed during the night, urinary amylase excretion was determined on both the 2-hour urine collection after dinner and the 24-hour collection in the patients with suspicion of pancreatic disease. Although the significant superiority of 2-hour collection over 24-hour collection was not confirmed, 2-hour collection was used both for the outpatients and the inpatients because of its convenience and sensitiveness as demonstrated in case 2.

**Urinary Amylase in Pancreatic Diseases:** The diagnostic significance of urinary amylase has also been studied by Gambill<sup>4</sup>, Viril<sup>32</sup>, and Budd<sup>3</sup> in carcinoma of the pancreas. Gambill<sup>4</sup> has demonstrated abnormal values in 27% of urinary amylase and in 13% of serum amylase observations. Above-normal values of urinary amylase were obtained about twice as often (56.2%) as of serum amylase (28.8%) in the present paper. The above-normal values

were obtained more frequently in association with pancreatic carcinoma of the head than with that of the body and tail, but in the cases of pancreatic carcinoma with liver metastasis, above-normal values were obtained less frequently and subnormal values (below the mean values minus a standard deviation) more frequently than in those without liver metastasis. These might have resulted from the following facts. The patients with carcinoma of the body and tail were diagnosed at the late stage of the disease and were associated with progressive malnutrition and destruction of the remaining pancreatic tissue and with liver metastasis. On the other hand, the patients with carcinoma of the head were often diagnosed at a little earlier stage and accompanied with ductal obstruction and chronic pancreatitis distal to the tumor. Further and more extensive studies are required on the correlation between the site or the extent of the lesion in the pancreas and the urinary amylase excretion.

The urinary amylase excretion is a highly sensitive indicator of the presence of acute pancreatitis. In the absence of renal insufficiency, the urinary excretion remained always abnormal when the serum concentration was abnormal, and the excretion remained abnormal for 2 to 50 days even after the serum concentration had returned to normal. Thus the urinary amylase excretion is a useful monitor for the diagnosis of subsiding pancreatitis and for convalescent care of acute pancreatitis, such as the initiation of oral feeding, the enrichment of dietary components, the discontinuation of bed rest, and the start of exercise.

In the patients with chronic pancreatitis the urinary amylase determination has also a significant diagnostic value as demonstrated by Kirschen<sup>25)</sup>, Viril<sup>32)</sup>, and Tierney<sup>33)</sup>. In the patients with acute exacerbation of chronic pancreatitis, the frequency of above-normal values of the excretion is high and as a matter of fact, such abnormal values were often obtained even in the cases at a relatively silent stage of chronic pancreatitis and were encouraging for the continuation of serial determinations of urinary amylase. Subnormal values of urinary amylase (below the mean values minus a standard deviation) were obtained more frequently in the cases of pancreatic calcification or fibrosis found at laparotomy, in which pancreatic exhaustion was highly suspected more than in those of other types of pancreatitis, nevertheless above-normal values of urinary amylase were observed in one-third of such cases. Tierney and his co-workers<sup>33)</sup> also observed the presence of abnormal urinary amylase in the patients with calcification of the pancreas. The relation between the excretion and the exhaustion of the pancreas are to be determined in future as mentioned before.

Urinary Amylase in Non-pancreatic Diseases: Clinical experience has shown that elevated serum and urinary amylase values are not pathognomonic of intrinsic pancreatic diseases, and hyperamylasemia and hyperamylasuria have been reported in such varied abdominal conditions as perforated peptic ulcer,



intestinal obstruction, and peritonitis. Hepato-biliary diseases also have been known to cause hyperamylasemia.

Above-normal values of urinary amylase were observed in some patients with biliary tract disease once or twice in a week during the serial determinations. These elevations might be due to unrecognized pancreatitis, as the biliary tract disease often accompanies its secondary pancreatitis.

Abnormally high urinary amylase values were observed also in some cases of acute or chronic hepatitis, or liver cirrhosis once or twice during the one-week observation. A similar study was performed on serum amylase by Cummins and Bockus<sup>34)</sup>. The above-normal urinary values seem to appear within one or two weeks after the onset of acute hepatitis and at the stage of marked hypertransaminasemia (SGOT and SGPT of over 500). The mechanism involved in the production of hyperamylasemia and hyperamylasuria in the hepatic disease is little understood. Amylase in the urine and the serum may be elevated due to increased release of amylase from the liver. This seems unlikely since the amylase activity was rather small in the human liver homogenate<sup>14)</sup>, and isoamylase of hepatic origin appeared only in a small amount in the urine of acute hepatitis<sup>35)</sup>. Another possibility is that the liver normally regulates the blood enzyme level by removing any excess. There are conflicting findings about this and final conclusion requires further studies. A third possibility is that increased hormonal stimuli to pancreatic secretion may result from decreased inactivation of hormones (Secretin, Pancreozymin) in the diseased liver, as pancreatic hypersecretion has been known in liver diseases<sup>36)</sup>. A fourth possibility is that hepatic disease may be associated with unrecognized pancreatitis. Interstitial fibrosis of the pancreas is often associated with liver cirrhosis. About a half of the cases of acute hepatitis with elevated serum amylase revealed the reduction of external pancreatic function stimulated by Pancreozymin and Secretin<sup>37)</sup>. These two support the fourth possibility. A fifth is that hemodynamic condition or permeability of the tissue may be affected by the diseased condition of the liver, and release of amylase from the pancreas may increase. Being confronted with many conflicting results, one concludes that the mechanism of increased serum and urine amylase in hepatic diseases is still unknown.

The elevated urinary amylase was observed also in some patients with peptic ulcer or functional disorders of the intestine. Unrecognized pancreatitis or penetration of ulcer to the pancreas can not always be denied in these cases.

Amylase retention in the serum in renal diseases has been well recognized with its decreased excretion<sup>38)~41)</sup>. The renal clearance of amylase was shown to be directly related to the creatinine clearance<sup>38)</sup>. Hyperamylasemia associated with renal diseases could be differentiated from other conditions by the determination of urinary amylase, serum creatinine, or blood urea nitrogen.

Urinary Amylase and the Data of Other Tests: The cases with abnormal

pancreatic secretion detected by the Pancreozymin-Secretin test corresponded well with the cases with abnormal urinary amylase. Although the relationship between abnormalities of urinary amylase and pancreatic secretion is to be clarified by further studies, the urinary amylase determination is to be performed as a screening test before the Pancreozymin-Secretin test for the diagnosis of pancreatic disease, since the latter is expensive and complicated.

When ductal obstruction or active inflammation or both are present in the gland still reserving adequately functioning tissue, then pancreatic stimulation could be expected to induce a significant rise in the serum amylase either through its regurgitation into the blood due to the obstruction of pancreatic flow or through the change in ductal permeability owing to the inflammation. Should disease of the pancreas have advanced to the stage in which little functioning tissue is left, no such increase in serum enzyme could be expected after pancreatic stimulation even though ductal obstruction is present or inflammation is still active. The positive reaction to the Vagostigmin (neostigmine) provocative test is well explained in the above-mentioned sequences, but the Vagostigmin test is considered to be not specific to pancreatic disorders as has been suspected. False results either for positive or negative are often encountered and the reproducibility of the test is rather discouraging. Despite the author's expectation, the abnormal response in the Vagostigmin (neostigmine) test was not significantly associated with the abnormal urine amylase excretion, though the mechanism for the abnormal results in the two tests is considered to be similar.

Normally the endocrine function of the pancreas is not affected by disease until extensive damage to the gland takes place, at which time an abnormal response to glucose tolerance test may be present. Contrary to the relationship between urinary amylase determination and Vagostigmin test, the result of urinary amylase excretion and glucose tolerance test showed unexpectedly significant correlation. Further studies are required on the relationship between pancreatic disease and genetic diabetes, or between the extent of damage to the pancreas and the endocrine function or the amylase excretion.

In the pancreatic diseases, jaundice and the elevation of serum alkaline phosphatase are often associated with abnormality of the biliary tract, and there is an additional possibility of the abnormality in the pancreatic duct from the close anatomical relationship of these two ducts. There was not observed significant a correlation between jaundice or alkaline phosphatase and the urinary amylase as expected, though the jaundiced patient with carcinoma in this study showed above-normal values of urinary amylase more frequently than nonicteric patients.

SGOT and SGPT showed no correlation with urinary amylase. The serum levels of GOT or GPT may be normal or elevated in patients with pancreatic disease. In the clinical, pathological, and laboratory study on 32 patients with

acute pancreatitis, Foulk and Fleisher<sup>42)</sup> found high GOT values in 22 patients, but they observed little relationship between the high values for the transaminase and the severity of the inflammatory lesions in the pancreas, and no correlation between the levels of serum amylase and lipase. In pancreatic carcinoma, serum transaminase levels are reported to be normal, unless there is metastasis to the liver or obstruction of the common bile duct.

High values of serum lactic dehydrogenase are ordinarily regarded as an index of seriousness of diseases, and in carcinoma of the pancreas suggesting the progressive destruction of the gland. In carcinoma, high values of this enzyme were correlated with high levels of amylase excretion, but not in pancreatitis.

Difference in Hyperamylasuria between Pancreatic and Non-pancreatic Diseases: In the 73 patients with pancreatic disease, the case ratios for abnormal urinary amylase excretion decreased from 56.2% to 34.3% and 45.3% according to the different denotation of abnormality by the values of over the mean values plus 2 standard deviations appearing at least twice a week, and over the mean values plus 3 standard deviations at least once a week, respectively. But this reduction in the case ratio was not statistically significant. In the 51 patients with non-pancreatic diseases, the case ratios were reduced likewise from 21.6% to 0.4% with a statistical significance of  $P < 0.01$ . Above-normal values (the mean values plus 2 standard deviations) and higher values (the mean values plus 3 standard deviations) were only rarely noted more than once and twice a week respectively in the patients without definite evidence of pancreatic disease. Thus, these criteria have contributed much in the differential diagnosis of pancreatic disease.

Isoamylase: The elevation in amylase activity observed in pancreatitis is consistently confined to the area where amylase of pancreatic origin has been assumed to belong<sup>13) - 17) 37) 43) - 50)</sup>. Elevated amylase activity of the  $\beta$ -globulin zone of electrophoresis in acute hepatitis accounted for about 7 to 12% of the total activity<sup>35)</sup>. The ratio of elevated hepatic amylase to the total activity is too far from causing hyperamylasemia during hepatitis due mainly to hepatic amylase. Many investigators have identified at least three different human isoamylases; from the liver, the pancreas, and the salivary gland<sup>37) 43) - 50)</sup>. All, however, have electrophoretic mobility similar to  $\gamma$ -globulin. Whether or not it is to distinguish pancreatic amylase from other organ amylase with similar patterns of electrophoretic mobility of  $\gamma$ -globulin zone, remains to be determined, and in this point lies the clinical usefulness of the electrophoretic separation of serum and urine amylase.

#### SUMMARY AND CONCLUSION

- 1) In order to determine the normal range of urinary amylase, 458 specimens

from 54 human controls were analysed using the Wohlgemuth method, with the result that the normal range was between 2 and 64 Wohlgemuth units. In about a half of the specimens there were observed values between 2 and 8 Wohlgemuth units which have been regarded as abnormally low.

2) Since the Wohlgemuth method is semi-quantitative, the results were compared with the amylase concentrations measured by Somogyi method. Wide overlaps were observed among the Wohlgemuth values expressed in Wohlgemuth unit. Wohlgemuth values, being influenced by urinary volume, are not valuable for the diagnosis of pancreatic diseases.

3) There were no significant difference among the mean values of amylase excretion per hour determined by Somogyi method in 2-hour urine before breakfast, after dinner, and in 24-hour urine. The upper limit of normal was 249 u/h or 5,016 u/day, but the lower limit could not be established.

4) Above-normal values of amylase were found in 28.8% of serum amylase, in 27.4% of urinary concentration, and in 56.2% of urinary excretion in 73 patients with pancreatic disease.

5) Urinary amylase excretion revealed a high diagnostic value in acute and chronic pancreatitis and in carcinoma of the pancreas.

6) Hyperamylasuria in non-pancreatic diseases was minor in degree and shorter in duration of its abnormality than that in pancreatic diseases. In non-pancreatic diseases, above-normal values of urinary amylase, appearing more than twice a week, were rare.

7) Elevated values of amylase excretion were observed more frequently in the patients with abnormal results of Pancreozymin-Secretin test, glucose tolerance test, and serum lactic dehydrogenase than in the patients without them. Significant relationship was not established between urinary amylase excretion, and jaundice, serum alkaline phosphatase, SGOT, SGPT or Vagostigmin (neostigmine) test.

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