

SOME OBSERVATIONS ON THE PATHOLOGIC PHYSIOLOGY AND THERAPY OF CHRONIC PANCREATITIS

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Experimental and clinical studies were carried out on exocrine, digestive and absorptive functions of dogs and human subjects with chronic pancreatitis by means of pancreozymin-secretin test, balance study and ¹³¹I-labeled meal absorption test. It seems reasonable to conclude that the failure of digestion plays a main role in chronic pancreatitis rather than that of absorption, and that the exhaustion extent of exocrine function can be measured fairly well with those tests.

Therapeutic effect of new pancreatic enzymes—FESTAL, COMBIZYM, and EIZYME—was estimated by new procedures. They seem to offer more efficient therapy to the malabsorption in the case of chronic pancreatitis.

In the condition of chronic pancreatitis, availability of carnitine therapy was evaluated in a sense of mild training. Further the correlation between therapeutic effect of the drug and the mechanism of its pancreatic stimulation as well as the various roles carnitine plays in the metabolism has been briefly discussed.

Pancreatic exocrine, digestive and absorptive functions have been investigated by employing new laboratory tests in order to clarify some problems on pathologic physiology of chronic pancreatitis. Pancreozymin-secretin test¹⁾²⁾ was adopted to determine the pancreatic secretion. As for digestion or absorption test, radioactive iodine tagged meal absorption test³⁾⁴⁾ and balance study have been used.⁵⁻⁷⁾ These laboratory procedures not only aim at the study of pathologic physiology of the disease, but also have a diagnostical value.

The treatment of chronic pancreatitis has three objectives⁸⁾: the first, palliation and termination of acute exacerbation; the second, prevention of further seizures and pancreatic destruction; and the third, control of external and internal pancreatic insufficiency. In essence, diet therapy has been settled in these two decades as the first choice in the treatment of the disease in any stadium. Based on the pathologic physiology of the condition, digestives

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(FESTAL, COMBIZYM, and EIZYME) and a pancreatic stimulant (CARNITINE) were used as remedies.

Clinical observation was carried out in thirty patients with chronic pancreatitis and eighty-nine patients with other digestive diseases who had been treated in our clinic during the past five years. The diagnosis was based on the history, physical findings, routine and special laboratory tests. In those tests were included blood or urine amylase determination, vagostigmin (prostigmin) test, *d*-xylose tolerance test, balance study and ¹³¹I-labeled fat, albumin and oleic acid absorption tests.

Pathologic Physiology

I. PANCREATIC EXOCRINE

Among many methods available in the study of pancreatic function, pancreozymin-secretin test involving estimation of the volume, lipase, amylase, and trypsin activities and bicarbonate content of the pancreatic secretion was employed.

METHOD

Estimation of Pancreatic Juice: Bartelheimer's "dreiläufige Doppelballonsonde" was used combined with pancreozymin-secretin test (Plate 1). The patient had been fasted overnight before the pancreozymin-secretin test and received no medication during the preceding day. Thirty minutes prior to the test 0.3 g of anaesthesin was given and the "sonde" was passed through the fasted patient under fluoroscopic observation until the top of the "sonde"

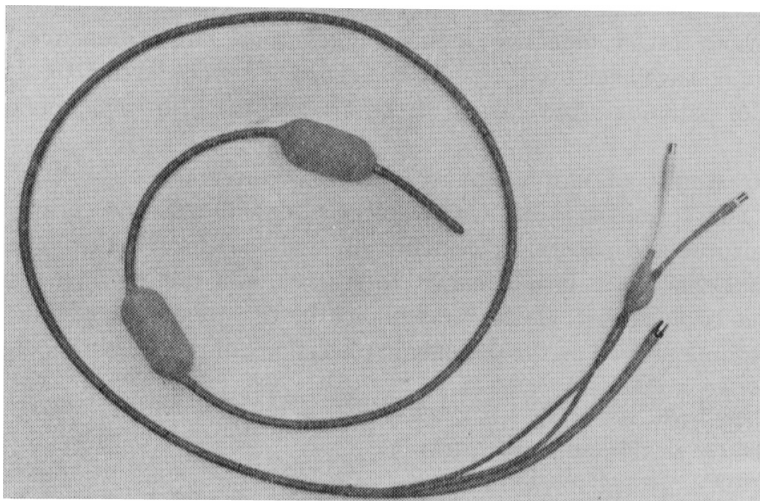


PLATE 1. Bartelheimer's dreiläufige doppelballonsonde.

reached the descendent loop of duodenum. After the duodenal juice became clear and alkaline in reaction, samples were collected as follows; one for 20 minutes before the stimulation and another for 10 minutes after the pan-creozymin (1 unit per kg body weight), then secretin injection was followed by 10-, 10-, 20-, and 20 minutes fractionated collections (1 unit per kg body weight). Test tubes for the collection of pancreatic juice contained small amount of liquid paraffin to cover the surface and were placed in a vessel with ice. The samples thus secured were measured for volume, and analyzed for enzyme and bicarbonate content. Lipase was determined by Cherry and Grandall's method, amylase by Somogyi's method, method, trypsin by Troll-Doubilet's method, and bicarbonate by the microdiffusion method of Conway. Pancreozymin and secretin used in this study were supplied from Boots Pure Drugs Company.

RESULTS

Contents of pancreatic juice: Results of quantitative determinations of the components of the pancreatic juice collected by "dreiläufige Doppelballonsonde" after the stimulation of pancreozymin and secretin from eighteen patients with chronic pancreatitis are given in Fig. 1. Mean values on normal controls were as follows: volume 313±98 ml; amylase $(2.28 \pm 0.98) \times 10^4$ u/ml; trypsin $(7.68 \pm 1.78) \times 10^5$ u/ml; lipase $(5.16 \pm 2.29) \times 10^2$ u/ml; and bicarbonate 4.07 ± 0.64 ml; (CO₂). The extents of reduction in volume, bicarbonate, content, and enzyme activity of pancreatic juice vary considerably among the patients, and more than two thirds of them showed apparently abnormal values. These evidences suggest that this method is one of a few procedures which are diagnostically useful.



FIG. 1. Duodenal contents obtained from patients with chronic pancreatitis by pancreozymin-secretintest. Thick lines: mean values for 13 normal controls with s.d.

II. DIGESTION AND ABSORPTION

The application of absorption test of ^{131}I -labeled triolein, albumin, oleic acid has been active lately. On the other side, the introduction of chromic oxide indicator method to the balance study has made the procedure of high value in the study of digestion and absorption. These methods were employed in this study.

1) ABSORPTION TEST OF ^{131}I -TAGGED MEAL

MATERIALS AND METHOD

Experimental dogs with chronic pancreatitis: Chronic pancreatitis was induced to dogs weighing 8 to 10 kg by infusion of olive oil into the main pancreatic ducts, with or without ligation of the ducts under anaesthesia with 20 ml of 2.5 per cent solution of sodium hexobarbiturate after an overnight fast. The dogs were used for the experiment 3 to 4 weeks after the operation.

Absorption of ^{131}I -labeled test meal: After three days' premedication with Lugol's solution (ten drops daily), radioactive iodinated test meal was given to the fasted dogs by tube, and to the patients orally. One ml of venous blood was drawn at hourly intervals and the radioactivity was measured by means of well type scintillation counter. The methods of making up test meals for the radioactive triolein and albumin absorption tests were given elsewhere,⁹⁾ and for oleic acid absorption test, a gelatin capsul containing 100 μc of ^{131}I -labeled oleic acid supplied from Dainabot R. I. Laboratory was used. Radioactivity in the entire blood of the body was calculated regarding the blood volume as 7.2 per cent of the body weight. The absorption rate of ^{131}I -tagged test meal was expressed in per cent of the dose administered.

RESULTS

Absorption of ^{131}I -labeled test meal: Fig. 2 and 3 represent the absorption rate of ^{131}I -labeled olive oil and albumin in experimental dogs of fifteen controls and eleven with chronic pancreatitis. The severity of disturbance varies considerably with dogs and it was found that when the test meal was the oil, two of the six cases with pancreatic impairment showed lower levels of blood radioisotope than the normal group. Moreover, all of the five cases showed lower level of blood radioisotope when examined with the protein test meal. As shown in Fig. 4, drastically decreased absorption rate of ^{131}I -labeled oleic acid in experimental dogs on the first or fourth day after the operation rapidly returned to the normal level in a week. Correspondingly, the elevated fecal excretion rate of radioisotope in the acute stage of pancreatitis returned to the normal level in a week just as shown in Fig. 5.

Fig. 6 shows the results in eight control subjects and seven patients with chronic pancreatitis tested with ^{131}I -labeled olive oil. All but one of the seven

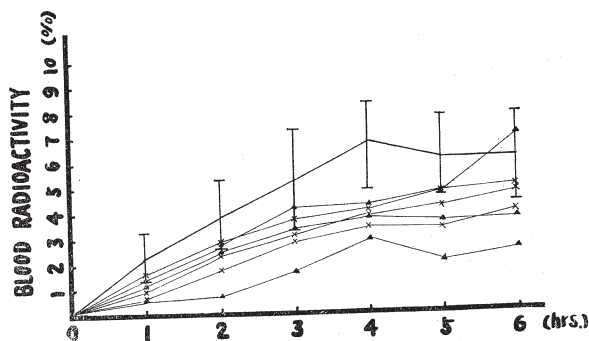


FIG. 2. Absorption curves of ^{131}I -labeled olive oil. Thick line connecting dots: normal dogs (average of 9 with s.d.); thin lines connecting triangles or crosses: dogs olive oil infused into the pancreatic ducts with or without ligation.

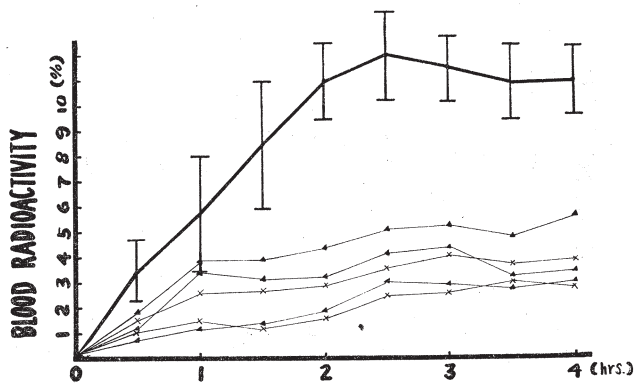


FIG. 3. Absorption curves of ^{131}I -labeled albumin. Thick line connecting dots: normal dogs (average of 9 with s.d.); thin lines connecting triangles or crosses: dogs olive oil infused into pancreatic ducts with or without ligation.

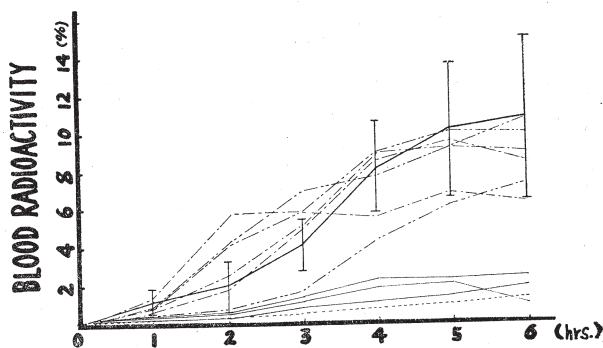


FIG. 4. Absorption curves of ^{131}I -labeled oleic acid. Thick line: normal dogs (average of 6 with s.d.); dotted line: 1st day; thin lines: 4th day; inverted lines: 7th day; double inverted lines: 3-4 weeks after production of pancreatitis.

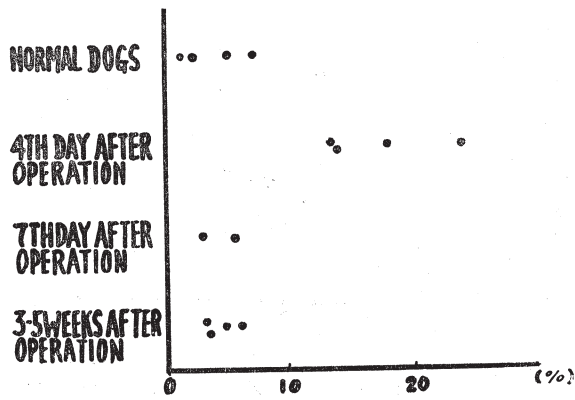


FIG. 5. Fecal excretion rate of ^{131}I -labeled oleic acid of experimental dogs.

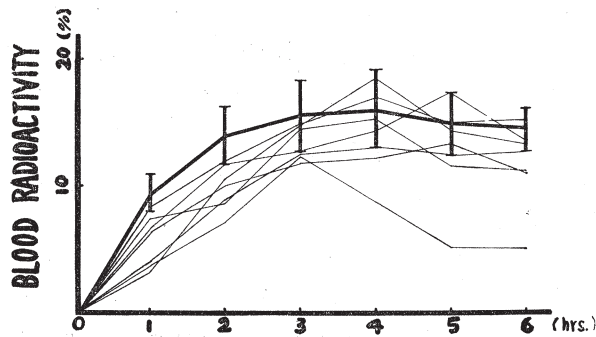


FIG. 6. Absorption curves of ^{131}I -labeled olive oil in patients with chronic pancreatitis. Thick line: normal (average of 8 cases with s.d.)

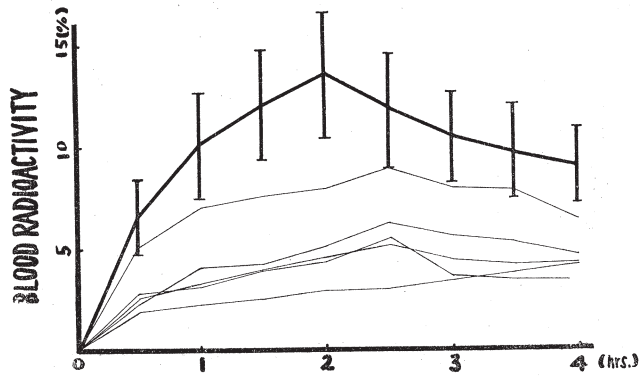


FIG. 7. Absorption curves of ^{131}I -labeled albumin in patients with chronic pancreatitis. Thick line: normal (average of 6 cases with s.d.)

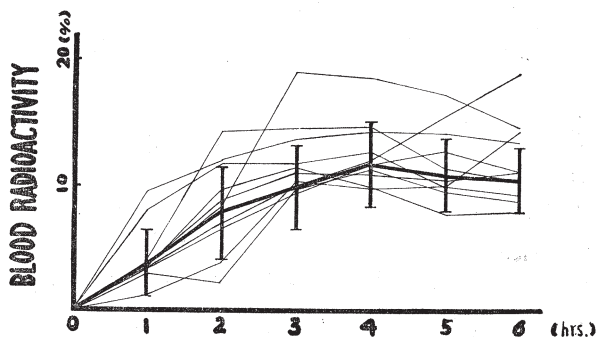


FIG. 8. Absorption curves of ¹³¹I-labeled oleic acid in patients with chronic pancreatitis. Thick line: normal (average of 4 cases with s.d.).

patients showed lower levels of blood radioisotope than the normal group. Fig. 7 indicates the results in six control subjects and five patients with chronic pancreatitis tested with ¹³¹I-labeled albumin. When protein was used as test material, the test of all the five patients resulted in definitely low blood radioisotope levels. Fig. 8 shows the results of four control subjects and ten patients with chronic pancreatitis tested with ¹³¹I-labeled oleic acid. It was seen that when oleic acid was the test meal all of ten patients showed blood levels of radioactivity approximately equal to those of normal controls. As for fecal excretion rate of ¹³¹I-labeled oleic acid, all of ten patients with chronic pancreatitis examined showed normal values (Fig. 9).

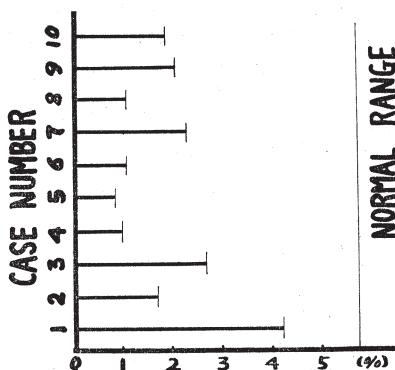


FIG. 9. Fecal excretion rate of ¹³¹I-labeled oleic acid in patients with chronic pancreatitis.

2) BALANCE STUDY

MATERIAL AND METHOD

The composition of the test meal used in the balance study is shown in Table 1. After three days' feeding with this diet, when the content of alimentary canal of the experimental subjects had been replaced by the test meal, the excreta was collected, dried at 70°C, crushed to powder, mixed thoroughly, and analyzed for Cr₂O₃ by Dansky-Hills method, crude fat by Saxon's method,

TABLE 1. Composition of Test Meal

| | |
|--------------------------------|------|
| Crude protein | 9.5% |
| Crude fat | 14.7 |
| Crude carbohydrate | 63.0 |
| Cr ₂ O ₃ | 0.1 |
| Others | 12.7 |

crude carbohydrate by Somogyi's method, and crude protein by Nesslerisation method. The calculation was done according to the following formulae⁵⁾:

$$\text{Total absorption coefficient (\%)} = \left(1 - \frac{A(100-B)}{B(100-A)}\right) \times 100$$

A: Cr₂O₃ content in test meal

B: Cr₂O₃ content in excreta

$$\text{Absorption coefficient of individual component (\%)} = \frac{a-b}{a} \times 100$$

a: ratio of individual component to Cr₂O₃ in test meal

b: ratio of individual component to Cr₂O₃ in excreta

RESULTS

Fig. 10 shows total absorption rate and absorption rates of protein, fat, and carbohydrate determined by Cr₂O₃ indicator method in three dogs with pancreatitis of either acute or chronic stage, and in five control dogs. It can be seen that the absorption rates of fat, protein, and total absorption rate are remarkably lowered by the operation, whereas that of carbohydrate is not

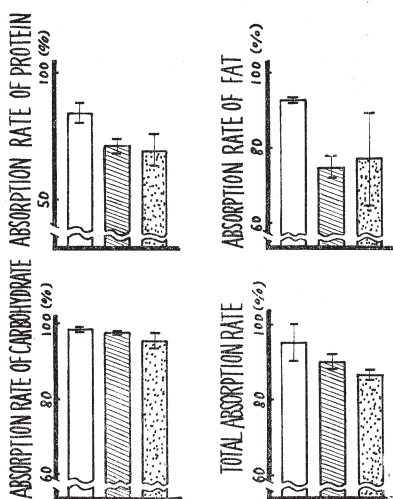


FIG. 10

FIG. 10. Absorption test with Cr₂O₃ indicator method.
 open column: normal dogs (average of 5 cases)
 hatched column: 4 days after the operation (average of 3 cases)
 stippled column: 3-5 weeks after the operation (average of 3 cases).

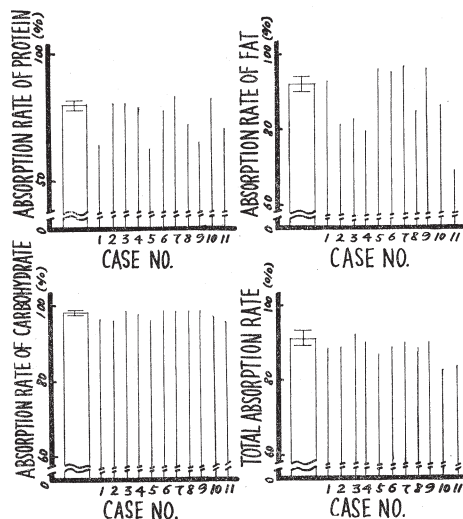


FIG. 11

FIG. 11. Absorption test with Cr₂O₃ indicator method in patients with chronic pancreatitis.

Open column: normal (average of 5 cases with s.d.).

different from that of normal. The decrease in the absorption rates is of the same order of magnitude between the acute and the chronic stadium of pancreatitis in these experimental dogs.

Fig. 11 shows the absorption rates in five control subjects and eleven patients with chronic pancreatitis tested by Cr_2O_3 indicator method. Some decrease in absorption rates of protein and fat were noted in four of the nine patients, whereas difference was scarcely seen between the patients and controls as to the total absorption rate and total absorption rate of carbohydrate. Digestive and adsorptive aspect of patients with chronic pancreatitis is obviously seen in Fig. 12, where the relationships between absorption rate of fat or protein and lipase or trypsin activity were evident, while absorption rate of carbohydrate in individual cases showed almost normal value irrespective of amylase activity.

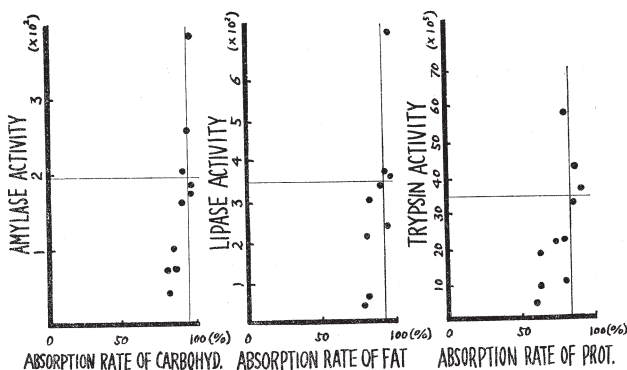


FIG. 12. Relation between enzyme activity and absorption rate in patients with chronic pancreatitis.

Pharmaceutical Therapy

As already shown, pancreatic secretion as well as digestion and absorption is generally disturbed in chronic pancreatitis. Above all, digestive failure evidently plays a main role in this circumstance. Therefore, therapeutic effect of digestives (FESTAL, COMBIZYM and EIZYME) and carnitine were studied, the former as replacement therapy, and the latter as a stimulant on the reserved function.

I. DIGESTIVE ENZYMES

MATERIALS AND METHOD

Medicaments used: The medicaments used were as follows: Pancreatin J. P., hitherto generally used crude enzyme prepare from swine pancreas; FESTAL, an enteric coated tablet comprising pancreatic lipase, pancreatic amylase, pancreatic protease, hemicellulase, and bile ingredients; COMBIZYM,

a sugar coated tablet composed of two layers—the external one contains vegetable enzymes (protease, amylase, cellulase, hemicellulase, and esterase), and internal one contains pancreatic preparations (lipase, trypsin and amylase); and EIZYME, an enteric film coated tablet containing pancreatic lipase, pancreatic protease, pancreatic amylase and cholic acid.

Dissolution test of the tablets: Dissolution test was done as follows: each tablet was placed in test tubes containing water, gastric juice (anacidic, normacidic and hyperacidic), and pancreatic juice (obtained with pancreozymin-secretin test, normal in either enzymatic activity or pH), respectively, incubated at 37°C in a water bath, shaken at ten minutes' intervals and the time was recorded when the tablet began to dissolve from the surface and when it disappeared.

RESULTS

Dissolving time of the tablets: Table 2 lists the laboratory data of dissolution time of FESTAL, COMBIZYM, and EIZYME. The results indicate the importance of the time of administration of these drugs with respect to the meal: FESTAL and COMBIZYM should be given simultaneously or immediately after the meal, and EIZYME, more than 30 minutes before the meal in order to get the greatest therapeutic effect.

TABLE 2. Dissolution Time of Drugs

| Medium→ Drug ↓ | Water (min) | | Gastric juice (min) | | | | | | Pancreatic juice (min) | |
|-------------------|--|-----|---------------------|-----|------------|-----|--------------|-----|---------------------------|-----|
| | | | anacidic | | normacidic | | hypereacidic | | | |
| | begin | end | begin | end | begin | end | begin | end | begin | end |
| FESTAL | 10 | 30 | 10 | 30 | 10 | 30 | 10 | 30 | 10 | 30 |
| COMBIZYM | 10 | 30 | 10 | 30 | 10 | 30 | 10 | 30 | 10 | 30 |
| EIZYME | no dissolution observed during 180 minutes | | | | | | | | 30 | 180 |

Incubated at 37°C, shaken every 10 minutes. Figures are the mean values of 10 experiments.

begin: beginning of dissolution of the surface of tablet.

end: disappearance of tablet.

Effect on absorption rate: Absorption test of ¹³¹I-labeled meal was done before and after the simultaneous administration of 1 g of pancreatin, 2 tablets of FESTAL or COMBIZYM in six dogs with chronic pancreatitis. As indicated in Figs. 13 and 14, a significant increase in absorption of ¹³¹I-labeled olive oil and albumin was seen in all cases.

Balance study was repeated in four dogs with chronic pancreatitis and two normal dogs as controls before and four days after the administration of

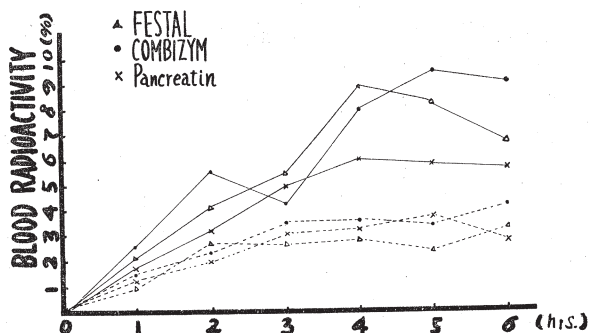


FIG. 13. Therapeutic effect of drugs on dogs with chronic pancreatitis observed with absorption test of ¹³¹I-labeled olive oil. Dotted lines: before; solid lines: after treatment. Mean curves for 4 cases.

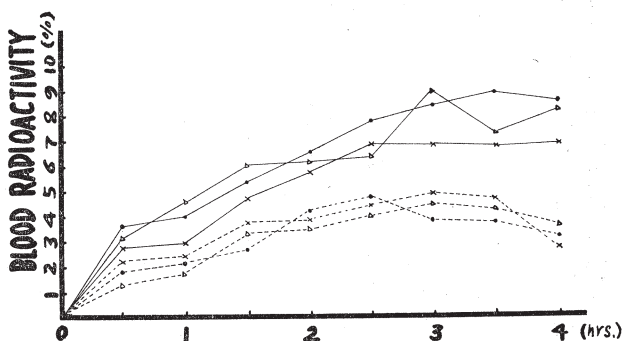


FIG. 14. Therapeutic effect of drugs on dogs with chronic pancreatitis observed with absorption test of ¹³¹I-labeled albumin. Marks and lines are the same as in Fig. 13. Mean curves for 4 cases.

EIZYME (2 tablets per day). The comparisons yielded the data shown in Fig. 15. In general, the lower the prior absorption rates were, the more marked improvements were obtained after the treatment.

With reference to the recommendation that for patients with chronic pancreatitis diets should be rich in protein, therapeutic effect of digestive enzymes (2 g of pancreatin, 2 tablets of FESTAL or COMBIZYM) on absorption of ¹³¹I-tagged albumin were observed in patients with chronic pancreatitis. The data are presented in Fig. 16. In all twelve patients with chronic pancreatitis an increased absorption rate was observed.

Therapeutic effect: Table 3 outlines the clinical effect of those drugs (as to carnitine, see below) in twenty-seven patients with chronic pancreatitis and seventy-one with digestive disease other than pancreatitis during 3 to 4

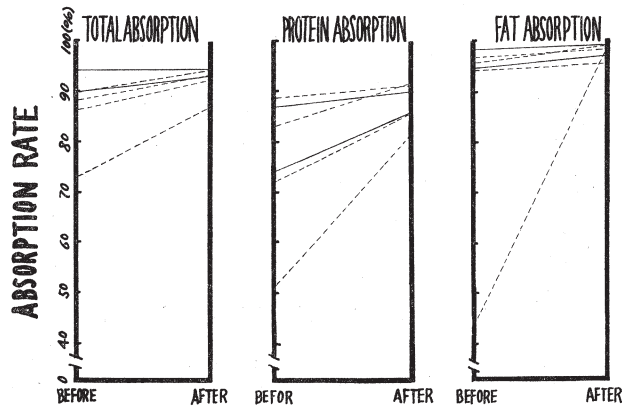


FIG. 15. Therapeutic effect of EIZYME on dogs with chronic pancreatitis tested with Cr_2O_3 indicator method. Solid lines: normal; inverted lines: diseased.

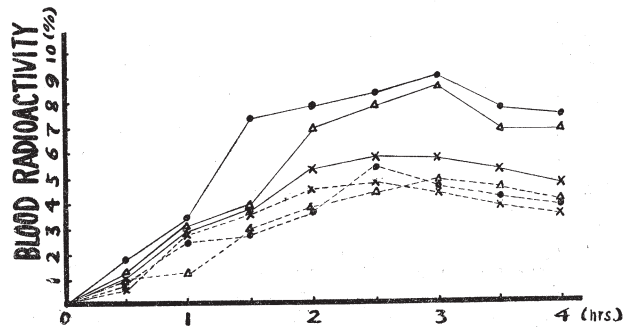


FIG. 16. Therapeutic effect of drugs on patients with chronic pancreatitis observed with absorption test of ^{131}I -labeled albumin. Marks and lines are the same as in Fig. 13. Mean curves for 4 cases.

TABLE 3. Therapeutic Effect on Symptom

| Drug | Case | Number | Appetite | | | Body Weight | | | Nature of stool | | | Side Effect |
|------------|------|--------|----------|----|---|-------------|----|---|-----------------|----|---|-------------|
| | | | ↗ | → | ↘ | ↗ | → | ↘ | ↗ | → | ↘ | |
| Pancreatin | A | 12 | 1 | 11 | 0 | 3 | 2 | 7 | 3 | 8 | 1 | 0 |
| | B | 8 | 3 | 5 | 0 | 2 | 4 | 2 | 1 | 7 | 0 | 0 |
| FESTAL | A | 4 | 2 | 2 | 0 | 3 | 0 | 1 | 2 | 2 | 0 | 0 |
| | B | 26 | 19 | 7 | 0 | 20 | 6 | 0 | 7 | 19 | 0 | 0 |
| COMBIZYM | A | 4 | 3 | 1 | 0 | 3 | 1 | 0 | 2 | 2 | 0 | 0 |
| | B | 10 | 8 | 1 | 1 | 7 | 3 | 0 | 6 | 4 | 0 | 0 |
| EIZYME | A | 7 | 3 | 4 | 0 | 2 | 4 | 1 | 5 | 2 | 0 | 0 |
| | B | 27 | 16 | 2 | 0 | 10 | 14 | 3 | 18 | 9 | 0 | 2 |
| Carnitine | A | 3 | 3 | 0 | 0 | 3 | 0 | 0 | 1 | 2 | 0 | 0 |
| | B | 18 | 11 | 6 | 1 | 11 | 7 | 0 | 3 | 15 | 0 | 0 |

A: Patients with chronic pancreatitis

B: Patients with gastrointestinal disease other than pancreatitis

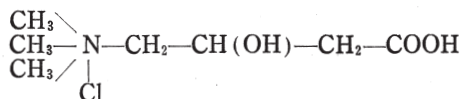
Side effect: nausea

weeks. Majority of the patients felt better; some apparently because of less diarrhea, some because of less gas, and others because of improved appetite and weight gain.

II. PANCREATIC STIMULATING AGENT
MATERIALS AND METHOD

For clinical investigation of the effect of carnitine (carnitine chloride) on pancreatic secretion, duodenal juice was collected by means of Einhorn's duodenal tube.

In the series of experimental dogs employed for the comparisons of the enzymatic activity and bicarbonate content in pancreatic juice, the operation to make up pancreatic fistula included the same technique as described before to approach the pancreas, and cannulation of a glass tube into the main pancreatic duct (variation of Boldgreff's method). The collection of the secretion was performed a week after the operation by the stimulation with adequate amount of carnitine.



RESULTS

Effect on pancreatic exocrine: Figs. 17-21 show the analytical values of pancreatic juice of dogs with pancreatic fistula after oral or intravenous administration of carnitine. Volume of the pancreatic secretion, as well as contents of amylase, lipase and trypsin was obviously increased in each case. The increase was prominent in cases stimulated by intravenous injection of 300 mg and stomach tube administration of 300 mg to 500 mg of carnitine. On the contrary, bicarbonate content showed a tendency to decrease in these

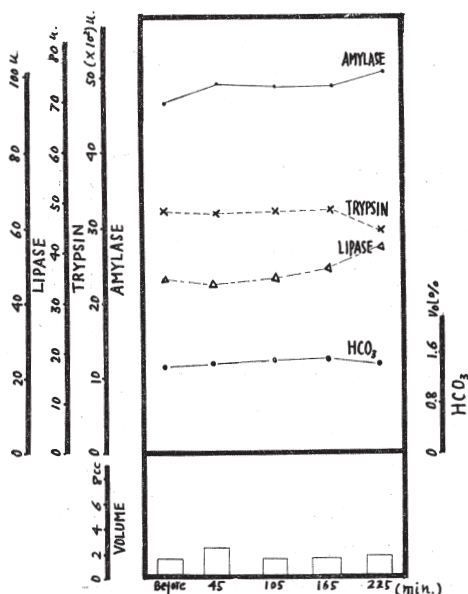


FIG. 17. Pancreatic juice obtained after intravenous injection of 100 mg of CARNITINE (dog).

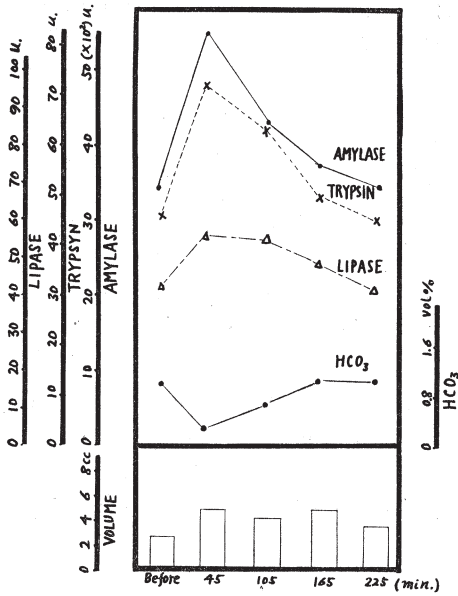


FIG. 18. Pancreatic juice obtained after intravenous injection of 200 mg of CARNITINE (dog).

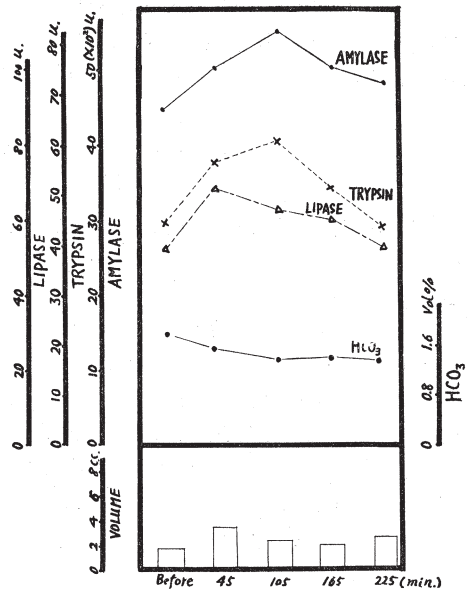


FIG. 19. Pancreatic juice obtained after intravenous injection of 300 mg of CARNITINE (dog).

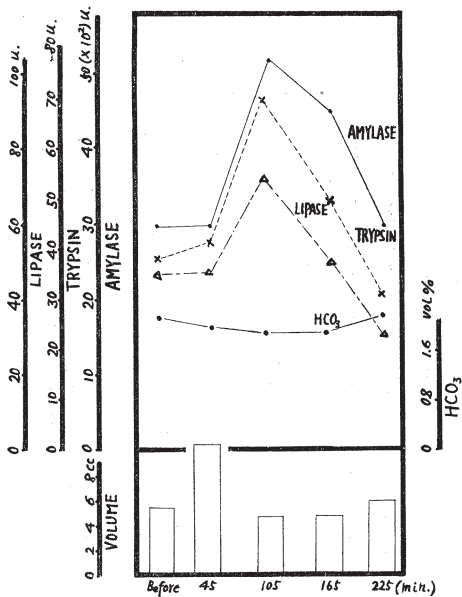


FIG. 20. Pancreatic juice obtained after oral administration of 300 mg of CARNITINE (dog).

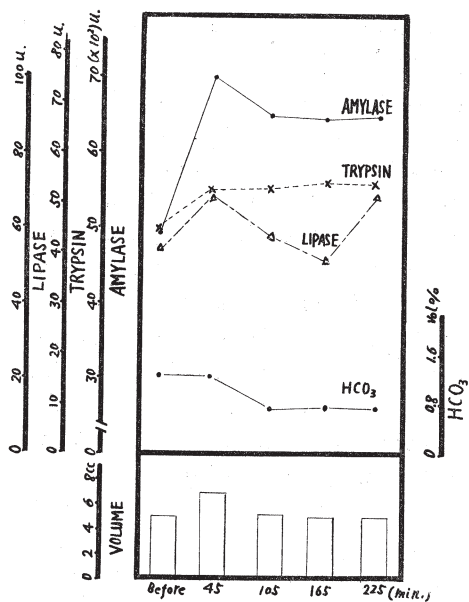


FIG. 21. Pancreatic juice obtained after oral administration of 500 mg of CARNITINE (dog).

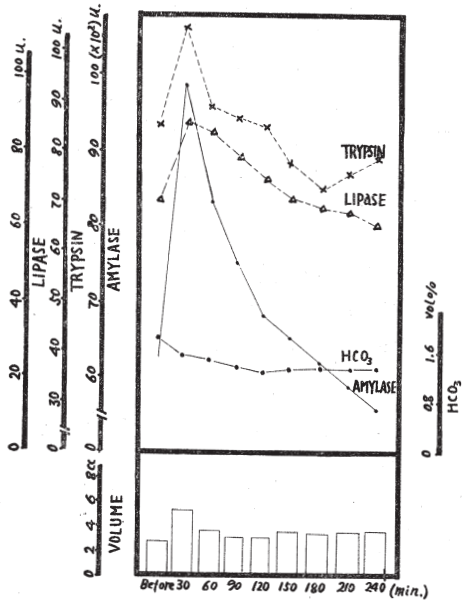


FIG. 22. Pancreatic juice obtained after intramuscular injection of 0.5 mg of vagostigmin (dog).

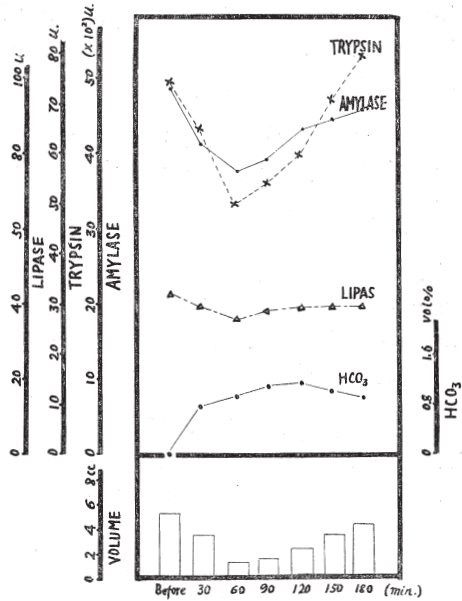


FIG. 23. Pancreatic juice obtained after intravenous injection of 300 mg of CARNITINE, premedicated with 0.1 mg of atropin (dog).

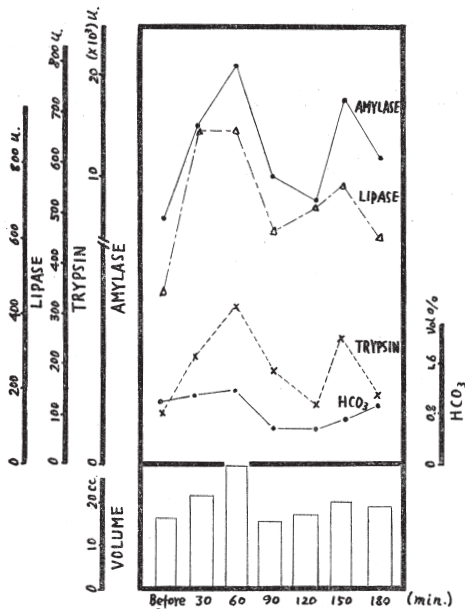


FIG. 24. Duodenal juice obtained after oral administration of 500 mg of CARNITINE from a patient with chronic pancreatitis (35-year-old male).

series. This type of secretion resembles that of vagostigmin stimulation (Fig. 22). These evidences strongly suggest vagus stimulating action of carnitine. With special reference to this, dogs with pancreatic fistula was premedicated with subcutaneous injection of 0.1 mg of atropine, and followed by intravenous injection of 300 mg of carnitine. As Fig. 23 presents, neither volume nor enzyme activity of pancreatic juice was increased. This means that atropine seems to antagonize the action of carnitine.

After the oral administration of 500 mg of carnitine, duodenal juice was collected from patients with chronic pancreatitis by means of Einhorn's duodenal tube, and was analysed for enzyme activity and

bicarbonate content. As an example (a case of 35-year-old male) is shown in Fig. 24, each enzyme activity and bicarbonate content showed a similar tendency to those in experimental animals.

Effect on absorption: As indicated in Figs. 25 and 26, absorption rates of ^{131}I -labeled olive oil and albumin were increased after 3 to 5 weeks' oral administration of 300 or 500 mg of carnitine to dogs with chronic pancreatitis.

^{131}I -labeled albumin absorption test was performed in three patients with chronic pancreatitis before and after the continuous oral administration of carnitine (500 mg per day) of 3 to 4 weeks. The comparison gave the data shown in Fig. 27. It can be seen that the carnitine administration produced a marked increase in blood radioactivity.

Therapeutic effect: The data on clinical observation are shown in Table 3. The patients including three with chronic pancreatitis and eighteen with gastrointestinal disorder other than pancreatitis appeared to feel better, es-

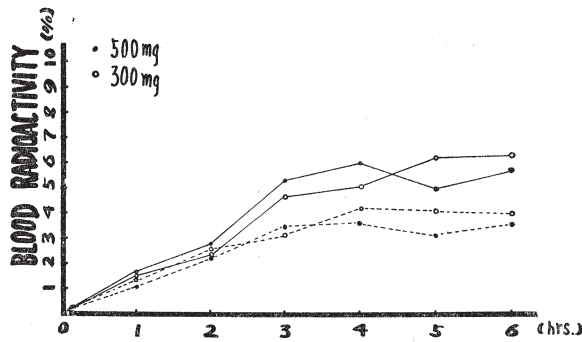


FIG. 25. Therapeutic effect of CARNITINE on dogs with chronic pancreatitis observed with absorption test of ^{131}I -labeled olive oil. Dotted lines: before; solid lines: after treatment.

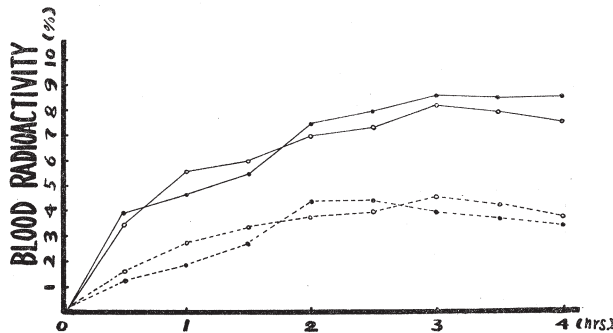


FIG. 26. Therapeutic effect of CARNITINE on dogs with chronic pancreatitis observed with absorption test of ^{131}I -labeled albumin. Marks and lines are the same as in Fig. 25.

pecially an improved appetite and a weight gain were characteristic.

Effect on blood sugar and serum amylase level: The determinations of serum amylase and blood sugar level were performed after oral administration of 200 mg of carnitine to the dogs, and 10 mg per kg of body weight to human subjects with reference to the vagostigmin-like action of carnitine to the pancreas. Fig. 28 shows the serum amylase levels after carnitine administration

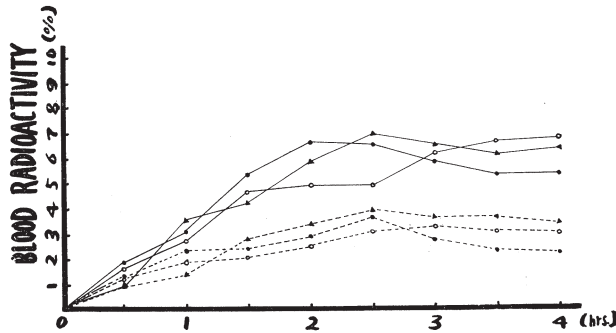


FIG. 27. Therapeutic effect of CARNITINE on 3 patients with chronic pancreatitis observed with absorption test of ¹³¹I-labeled albumin. Dotted lines: before; solid lines: after treatment.

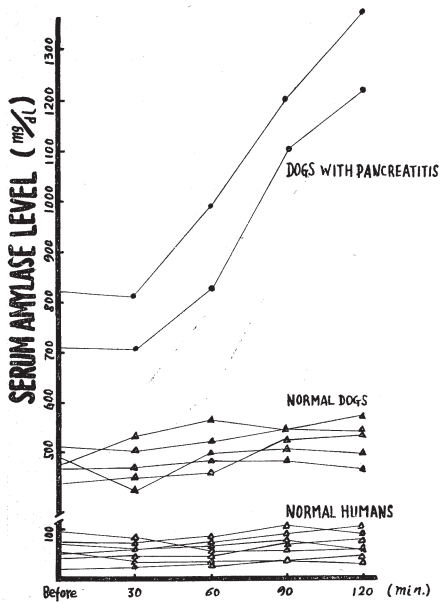


FIG. 28. Effect of CARNITINE on blood amylase level.

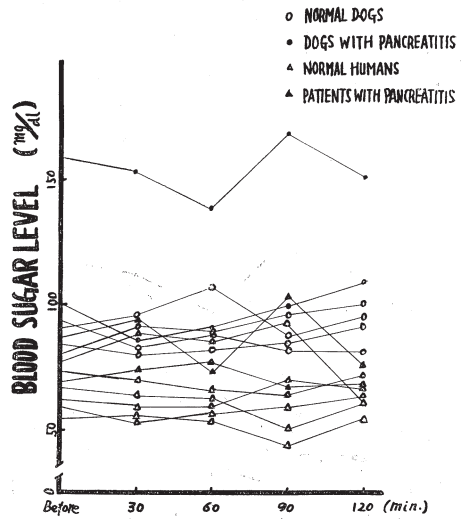


FIG. 29. Effect of CARNITINE on blood sugar level.

in six normal humans, five normal dogs, and two dogs with chronic pancreatitis. In normal subjects carnitine injection produced no significant increase in serum amylase. On the contrary, the values on dogs with chronic pancreatitis agreed well with those expected to be obtained by vagostigmin test. The result of blood sugar determination after carnitine administration in six normal humans and two patients with chronic pancreatitis is shown in Fig. 29, in which carnitine gave little effect in all cases.

Effect of carnitine on the ultrastructure of pancreatic acinar cell: To investigate the effect of carnitine on the ultrastructure of the pancreas by means of electron microscopy, guinea pigs weighed 250 to 300 g were used (For further details on the method, refer to Suzuki's report¹⁰).

Specimens of pancreas were taken from the guinea pig, 30 minutes after the intramuscular injection of 100 mg of carnitine, and 90 minutes after oral administration of 200 mg of carnitine for the ultramicroscopic study (Plates 2 and 3). Sections revealed zymogen granules in pancreatic acinar cells generally increased in number: which clearly indicates an active state of the cell. Mitochondria in part revealed the destruction of both double layered structure of limiting membrane and cristae mitochondriales. The endoplasmic reticulum

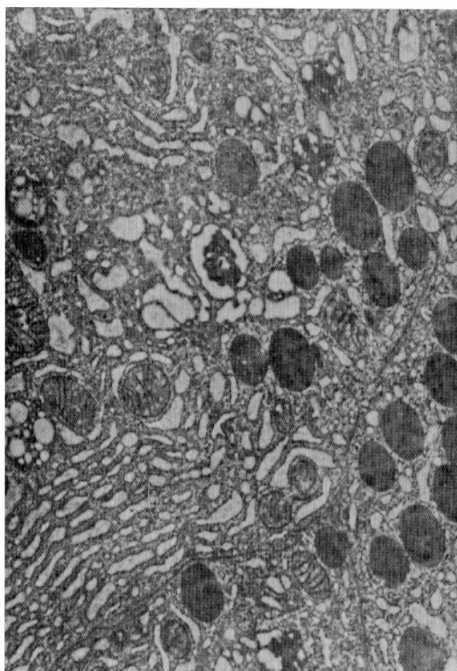


PLATE 2. Electron microgram of guinea-pig pancreas, 30 min. after intra muscular injection of 100 mg of CARNITINE.

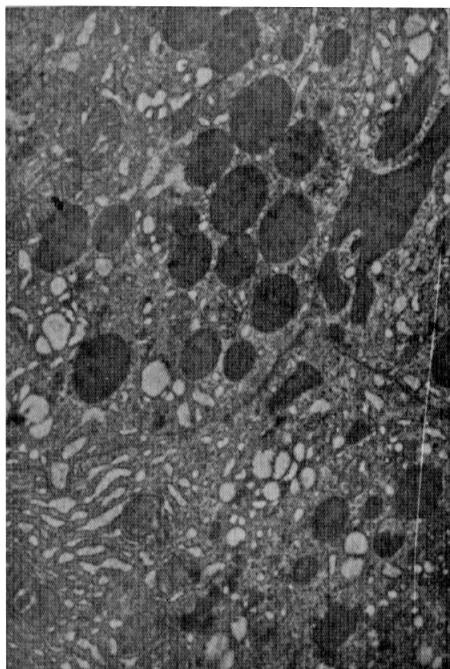


PLATE 3. Electron microgram of guinea-pig pancreas, 90 min. after oral administration of 200 mg of CARNITINE.

showed a marked decrease and formed networks in some areas. As a whole, the decrease of the endoplasmic reticulum was more marked with carnitine than that obtained with vagostigmin injection, except that the small particulate component containing Palade's particle was more markedly reduced with carnitine. On the other hand, Golgi complex, especially Golgi vacuole, developed a great deal. The extent to which the development took place was greater than that obtained by vagostigmin injection. However, there was no parallelism of increase between so-called intravacuolar granule and Golgi vacuole. Cervic formation in cytoplasm, the content of which is considered to be secretory granule, was also noticed in the case of carnitine stimulation as it was noticed in the case of vagostigmin stimulation.

DISCUSSION

There have been many divergent views on the possible value of the pancreozymin and secretin test.¹¹⁾⁻¹⁸⁾ If ductal obstruction or active inflammation is present in a gland still containing adequate functional tissue, stimulation might be expected to induce a significant rise in serum enzymes or reduction in duodenal contents. However, should disease of the pancreas have advanced to the point where little functioning tissue is left, no such effect on serum enzyme could be expected after pancreatic stimulation, even if ductal obstruction is present and inflammation is still active. Keeping in mind the frequently presented obstructive lesions in chronic pancreatitis, administration of pancreozymin first to sweep out preformed enzymes from the acini into the ductules followed shortly after by secretin to increase the volume of secretion should be theoretically the best procedure for collection of enzymes in such a case. For this reason administration of pancreozymin was carried out first, followed in 10 minutes by secretin¹⁾ rather than the reverse order and the longer intervals of 30 to 60 minutes between the hormone injection used by Howat.¹⁹⁾

In order to collect pancreatic juice as perfectly as possible without contamination of gastric juice, revised methods have been forwarded, among which "dreiläufige Doppelballonsonde" seems to be the best at present. The utility of the "sonde" was shown by the detailed experiment of Müller-Wieland.²⁾ "Dreiläufige Doppelballonsonde" was used for the first time in combination with the stimulation by pancreozymin and secretin in this study. The severity of pancreatic dysfunction is considerably various in the patients, and more than two thirds of them showed lower levels of pancreatic contents. It is interesting that normal value of amylase obtained in this study was ten times as much as that reported by Sun and Shay.⁸⁾ This fact might be attributed to the difference of race, or to the difference of dietary composition, which, according to Grossman's experiment,²⁰⁾ has an influence on production of pancreatic enzymes. Considering that this procedure of pancreozymin-

secretin test with analysis of duodenal content concerns directly with pancreatic exocrine function, these results are of much importance, and the procedure can be considered as diagnostically useful.

As for digestion and absorption tests, absorption test of ^{131}I -tagged meal and balance study with indicator of Cr_2O_3 were adopted. The clinical use of radioactive iodinated fat was initiated by Tanhauser and Stanley in 1949. A number of papers published since that time have gradually led to the development of several simple clinical procedures using ^{131}I -labeled triolein for the study of fat absorption. Reemtsma and coworkers⁴⁾ pointed out the usefulness of ^{131}I -labeled oleic acid in conjunction with ^{131}I -labeled triolein in differentiating absorptive from digestive defect. However, Malm *et al.*²¹⁾ and Isley *et al.*²²⁾ found that the blood radioactivity following the ingestion of the tagged fatty acid in normal subjects was somewhat lower than that of the tagged whole fat. On the other hand, Kaplan *et al.*²³⁾ found no difference between fat and fatty acid absorption in their normal controls. One should expect no difference according to Verzar's lipolytic theory of fat absorption, in which the fat is supposed to be broken down entirely into fatty acid prior to absorption. According to Frazer's "partition hypothesis"²⁴⁾—emulsification in the upper part of intestine normally yields particles of $0.5\ \mu$ or less in diameter, and in this form they can pass through the intestinal barrier without molecular alteration—absorption of neutral fat and partly hydrolyzed fats, diglyceride or monoglycerides, occurs in the intestine; this fact might account for lower fatty acid absorption. Absorption test of ^{131}I -labeled albumin was initially carried out by Lavik in 1952. It was highly evaluated by Chinn and Shingleton in the diagnosis of pancreatic cancer and chronic pancreatitis. However, it is not so widely used as radioactive iodine tagged triolein because of its lower rate of accuracy. Many types of investigations have been carried out in searching the best method for the absorption test of ^{131}I -tagged meal. Blood, serum, serum protein, serum lipid, and feces were used as samples. Masuda²⁵⁾ and McKenna²⁶⁾ preferred to take excretion rate in feces to absorption rate in blood. But the blood has been most frequently used owing primarily to the simplicity of procedure. Goldman and Jordan²⁷⁾ reported that the measurement of radioactivity in blood was evidently better or at least no worse than the other methods. According to the criteria of Beskowitz and Schlaroff,²⁸⁾ attention should be paid to the following points: 1) summation of the absorption rate at 4-, 5-, and 6 hours after the administration; 2) the time necessary to reach the peak value; 3) the highest absorption rate; 4) disappearance rate in blood.

In the presented data, ^{131}I -labeled olive oil and albumin absorption rate in the dogs and patients with chronic pancreatitis were apparently lowered satisfying the first of the above criteria, which is most often used. As for

the absorption of ^{131}I -labeled oleic acid, it was extremely lowered shortly after the operation and recovery could be swiftly seen within seven days, and reached the same level as the normal controls after 3 to 5 weeks. High excretion rates shown at the early stage of pancreatic impairment were returned to normal in the corresponding period. In the clinical study on patients with chronic pancreatitis, both ^{131}I -oleic acid absorption rate in blood and excretion rate in feces showed no difference from those of normal subjects. The evidence strongly suggests that the disturbed absorption mechanism in intestinal mucosa at the acute stage of pancreatitis (histological and histochemical study for this was reported by Fujimoto²⁹) is improved to be undetectable when acute inflammation is ceased, and disturbance in absorption can be excluded in chronic stadium of pancreatitis.

In stead of total collection method which includes complicate procedure and inaccuracy in result, indicator methods have been introduced to the balance study. Iron, animal charcoal, silica, and chromic oxide were examined as indicators. According to the reports of Yanagizawa⁷) and Schurch⁶), chromic oxide is the most suitable for the test, because it is quite harmless, neither digested nor absorbed, gives no disturbance to the physiological state of intestine, and it can be mixed homogeneously with diet and passes through the intestinal canal without being denatured. The results in this study are essentially similar to those of Yamagata³⁰) in which forty per cent of patients with chronic pancreatitis showed more or less abnormality. The relationship between absorption rate and duodenal contents is that the absorption rate of carbohydrate is almost normal irrespective of lowered amylase activity, while decreased absorption rates of fat and protein are to a certain extent proportional to lipase and trypsin activity. These evidences suggest that the digestion of carbohydrate is well compensated by the enzyme in saliva, intestinal juice and others. As clearly seen in the data presented, there are marked destruction of the pancreas before the excretion insufficiency manifested, which has been merely speculated.

Thus in chronic pancreatitis, the disturbance in absorption is chiefly attributed to diminished pancreatic secretion. While pancreatic enzyme therapy is essential to substitute for the pancreatic impairment, fundamental problems on its pharmaceuticals and prescription have not been fully settled, chiefly because of difficulty in clarifying the dynamic aspect of digestion and absorption. It is regrettable that the clinical use of the enzymes seems to be rather habitual or routine. Discussion of substitutional therapy constitutes two main problems, one is property of enzyme itself and the other its availability in the digestion mechanism.

With the modern advances in enzymic chemistry, excellent digestive enzymes have been brought to clinical use. For example, *Aspergillus niger*

when cultivated in acid medium produces much of amylo 1-, 6-glucosidase (Cori), and when cultivated first in acid and then in neutral medium produces acid resistant alpha-amylase. Likewise, a new acid resistant protease obtained from *Asp. saitoi* which can be activated without any metal ion.³²⁾ Patients with chronic pancreatitis generally show disturbed secretion of gastric juice but even in normal individuals frequent changes in values of pH in gastric content after an ordinary meal have been reported to range from 4.0 to 5.3, after 30 minutes, from 3.5 to 5.0, after 60 to 90 minutes, and from 3.5 to 5.0 after 60 to 90 minutes.³¹⁾ In these conditions the acid resistant digestive enzymes from microorganisms are effective even without coating: those are TAKA-diastrase from *Asp. oryzae* (optimal pH 3.7-6.5), Vernase from *Asp. oryzae* TRP-18 (optimal pH 2.7-6.0), Sanactase from *Asp. niger* (optimal pH 2.5-4.5), Acidase from *Asp. awamori* (optimal pH 4.5-5.0), Molcin from *Asp. saitoi* (optimal pH 2.7-3.0), and Tetrase from *Rhizopus chininsis* (optimal pH 4.5). Moreover, combined administration of 0.05 g of pancreatin or Taka-diastrase (alpha-amylase) and 0.05 g of diastase (beta-amylase from yeast) was reported to be more effective than 0.3 g of diastase alone. Similar potentiations among pancreatin, diastase, pepsin (from hog pancreas), Taka-diastrase and molcin were reported.³²⁾ The data of dissolution test presented here disagree with those by Kanig³³⁾ in which dissolution time of seventeen kinds of tablets of commercial products varied from 15 minutes to 4 hours, even those of the identical lot number. This indicates the difficulty of the procedure of enteric coating. There have been a few roentgenological studies on retention of tablets in stomach. Crane³⁴⁾ found that in fifteen per cent of cases the tablets stayed in stomach for 9 to 10 hours after the administration. Inuma³¹⁾ reported that in twenty per cent of a hundred cases tablets were found to stay in the stomach for as long as 4 hours. To prevent the stagnancy, Okazaki³²⁾ recommended a gastric film coated capsul containing two kinds of granules, the one is naked granules of enzymes active in gastric juice, the other those with enteric coated.

Administration schedule is also an important factor for the efficacy of substitution therapy. Three times a day after each meal has been the usual method, but according to the opinion of Grossman³⁵⁾ or Heinsen³⁶⁾ the medicament is to be given not only after meals but also before and between meals. It is based on the assumption that the constant presence of the digestive enzymes in the alimentary tract prevents stimulation of the pancreatic secretion which is favorable to keep rest of the pancreas. This problem is not investigated in this study, but Jordan *et al.*³⁷⁾ reported that 8 g of Viocase (preparation from swine pancreas) was obviously more effective when given 12 times hourly (0.66 g each), than when given in 3 divided dosages (2.7 g each), to patients with pancreatic insufficiency.

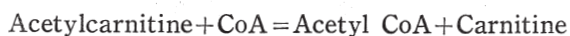
As for the doses, Henning *et al.*³⁸⁾ in the examination of patients with complete "Pankreas achilie" by means of test drink through duodenal tube, reported daily demands of digestive enzymes facilitating complete compensation: tripsin demand corresponded to the dose to liberate 360 mg of beta-naphthylamine; lipase, 240 g of beta-naphthol; chymotrypsin, 19.2 g (!) of beta-naphthylamine; carboxypeptidase, 480 mg of beta-naphthol; amylase, 4 800 g (!) of maltase, respectively. According to the data the daily doses were calculated to be as much as a hundred tablets or more of those we use generally (*e.g.*, 250 g of COMBIZYM or 615 g of FESTAL is the daily dose of lipase or chymotrypsin replacement). Yamagata also reported on a pancreatectomized patient whose diarrhea was subsided only under the administration of 30 g of pancreatin.³⁹⁾ Of course, the doses of supplement varies with cases, and few patients with chronic pancreatitis generally needed such a large amount of digestives. This is explained by the compensation of enzymes from other organs and intestinal flora, functional reserve of pancreas, diet therapy (large amount of fat is forbidden), and potentiation effect of enzymes, as the improvement of enzymatic activity in duodenal juice as a result of initial replacement therapy. The evidences suggest that the introduction of these new digestive enzymes may lead to a more logical therapeutic regimen.

Since the discovery of carnitine, a number of experimental and clinical studies have been done on its effects on various organs.³⁹⁾⁻⁴¹⁾ Among them, the effect of the substance on digestive system and metabolism has attracted much interest. As already described, carnitine stimulates parasympathetic nerves and promotes digestive function. Admitting considerable variation in susceptibility of individual dogs, intravenous administration of 1 to 20 mg per kg body weight of carnitine caused remarkable salivation, and premedication of atropine depressed the effect. Concentrations of hydrochloric acid and pepsin in gastric juice were also increased in both dogs and human being. An increase from 150 to 300 per cent in bile secretion was also obtained with intravenous injection of 1 to 20 mg per kg body weight of bicarnesine (carnitine carnitate) in dogs but the effect could not be observed with atropine injection.⁴²⁾ The presented findings of intense effect of carnitine to stimulate pancreatic exocrine function without exception are essentially similar to those of Albert *et al.*⁴³⁾ Binon and Deltour⁴⁴⁾ compared exocrine stimulating action of secretin with that of carnitine and found the superiority of the latter in volume and dried weight of secretion. They attributed this finding not only to the stimulation of parasympathetic system but also to a specific pancreotropic property of carnitine itself. Obvious improvement of ¹³¹I-tagged test meal absorption was observed in the cases treated with carnitine in this study. Considering the effect of carnitine on various digestive organs, it is reasonable to assume that such improvement was due not only to agitated pancreatic

exocrine function but also to a stimulation of the alimentary system as a whole. In the present study, no significant change was observed in blood sugar level with carnitine administration, but different opinions on its effect of lowering blood sugar level have been presented. The report that bicarnesine reduces the insulin demand of patients with diabetes mellitus seems to suggest some roles played by this drug in the sugar metabolism.⁴⁵⁾

Similarity in their chemical structures (replacement of a hydrogen atom in OH radical of choline by CH_2COOH makes carnitine) called attention to mutual exchangeability between choline and carnitine in nutrition and metabolism. In the insects for whose development carnitine is an essential factor, choline could not completely compensate the carnitine demand, but no report has been made on whether choline can reduce the demand of carnitine. Inversely, the compensation of choline demand by carnitine has been proved in some organism.⁴⁶⁾ Because of its similarity in chemical structure with choline and betaine, methylating action of carnitine has also been expected and investigated. Ciusa and Nebbia⁴⁷⁾ first investigated on this problem in human subjects and reported that carnitine acts as methyl donor in the intermediate metabolism of thiamine. But the controversy continued as to whether such action as methylation of carnitine exists.⁴⁸⁾ The rise of serum amylase level was evidently induced with carnitine administration to dogs with pancreatic impairment in this study. This effect of parasympathetic stimulation raised a question as to whether the effect was based on the similarity to choline or on the anti-cholinesterase action as that of vagostigmin. Further investigations on this problem are necessary.

There are some interesting studies on the role of this substance in fat metabolism. MacFarlane⁴⁹⁾ and Bekaert⁴⁵⁾ suggested fat catabolic action of carnitine in *Tenebrio* and patients with diabetes mellitus showing lipemia. Fritz⁵⁰⁾ observed that an extract of muscle was able to accelerate parmitic acid oxidation, and identified this accelerator with carnitine. The quaternary ammonium base of phospholipid in serum has been identified with choline. But Binon and Deltour⁵¹⁾ showed that one third of it was carnitine and they suggested that carnitine should take an important part in regulation of the serum lipid level in the form of phospholipid. Friedman *et al.*⁵²⁾ found an enzyme in the liver of pigeon and sheep which catalyzes the reaction described below.



The important role acetyl CoA plays in TCA cycle is well known. As a rule, it is impossible for acetyl compounds with low energy bond to donate acetyl radical to CoA except for a few ones. Therefore this character of acetylcarnitine is to be highly evaluated. Acetylcarnitine has not yet been found

in any organism but Fraenkel⁵³⁾ asserts that carnitine acts in the form of acetylcarnitine.

Suzuki⁵⁴⁾ previously compared the patterns of pancreatic secretion with secretin and vagostigmin stimulation in his study on ultra structure of guinea pig pancreas. In general, the pattern obtained after carnitine injection in this study resembled that with vagostigmin, but detailed examination revealed that development of Golgi vacuole, increase in zymogen granules and decrease in endoplasmic reticulum surpassed those with vagostigmin injection, which supports the concept of those authors on carnitine.

Although a number of publications on clinical use of have appeared in recent years, there are few which concern to chronic pancreatitis. The author's observation, though it was for a comparatively brief period with a small number of patients, indicates that the drug can be an adjuvant in the treatment of chronic pancreatitis in which the primary disturbance is in the secretion of enzymes. With its general stimulating effect on digestive system and some favorable effect on intermediate metabolism of various substances, carnitine therapy is especially recommended in the sense of mild training for patients with chronic pancreatitis who are in remission and retain functional reserve.

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