Trypsin Release, Kinin Production, and Shock

Relationship in Experimental and Human Pancreatitis

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As early as 1916 Peterson [1] suggested that the fulminating nature of acute hemorrhagic pancreatitis was directly related to the digestion of plasma proteins by proteolytic enzymes. In 1950, Werle [2] published his classic work on the hypotensive proteolytic material from the pancreas, kallikrein, and more recently, kallikrein was partially purified by Westerfield [3] and Webster and Clark [4] and shown to produce hypotension. Rocha e Silva,[5,6] after demonstrating the ability of trypsin to release bound stores of histamine, discovered that trypsin also released from plasma α-2 globulins the hypotensive polypeptide bradykinin in much the same fashion as renin liberates the hypertensive polypeptide angiotensin. The object of the present investigation is to measure the liberation of proteolytic enzymes in pancreatitis and to relate this to the release of substances affecting vascular smooth muscle and capillary permeability.

Materials and Methods

1. Experimental Pancreatitis.—Healthy, adult mongrel dogs, ranging in weight from 15 to 20 kg were anesthetized with intravenous pentobarbital. All dogs had been fasted overnight. Through a vertical midline incision, the duodenum was mobilized; the accessory pancreatic duct (major duct in the dog) was isolated as it entered the duodenum. A small incision was made, and a No. 60 polyethylene catheter was introduced a few millimeters and secured with one 3-0 silk tie. Ten milliliters of bile from the dog's own gallbladder or 10 ml of bile pooled from all the dogs used on that day were injected into the cannula under moderate pressure. Usually the body and tail were injected and occasionally the uncinate process as well. This form of pancreatitis was rapidly fatal to all animals within 24 hours.

2. Pancreaticoduodenectomy.—Total pancreaticoduodenectomies were performed on 25 dogs at various intervals after bile-induced pancreatitis in the following manner: After the allotted time, the vessels to the uncinate process and the mesentery were clamped; the distal duodenum was cut between clamps at the level of the uncinate process. The arterial and venous supply to the pancreas was ligated. The duodenum was next divided just below the pylorus. The branches of the splenic artery and vein to the tail of the pancreas were next isolated and ligated, and the entire pancreas and the duodenum were removed from the abdomen. Bowel continuity was restored by gastroenterostomy, and the gallbladder was drained into the stomach. Before the abdomen was closed, the peritoneal cavity was washed out with 1,000 cc of sterile saline. The latter step is very important as will be seen by the data to follow. The entire resection takes less than five minutes and can be accomplished with little blood loss or trauma.

3. Measurement of Trypsin Activity.—Trypsin was assayed by measuring the rate of hydrolytic activity on a 1 mM solution of TAMe (a-p-toluenesulphonyl-L-arginine methyl ester HCl) in a 0.01 mM tromethamine buffer at pH 8.0±0.5. Known amounts of the test solution (0.1-0.5 ml) of suitable dilution, were added to 3 ml aliquots of the substrate, and the reaction was allowed to proceed at 37 C for periods of either 5, 10, or 15 minutes depending on the concentration of trypsin present. The reaction was stopped by the addition of 0.2 mg of soybean trypsin inhibitor (SBTI) and the samples were then centrifuged under refrigeration at a speed sufficient to remove all cloudiness. Blanks on which the tests measured were prepared by placing SBTI in the substrate prior to the test solution. All assays were performed in duplicate and measured in a Beckman DU spectrophotometer at a wave length of 247μ. The method,[7] a modification of that of Hummel, gave highly reproducible results.

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4. Vasoactive Substances.—Histamine, serotonin, catecholamines, and polypeptides were identified and measured by the bioassay previously described using the technique of sequential blocking of an arterial strip with pyrilamine maleate, lysergic acid diethylamide (LSD*) and phenoxybenzamine hydrochloride (Dibenzyline),† respectively.[8,11]

Experimental Results

1. The Effect of Pancreatectomy on the Course of Pancreatitis.—Pancreatectomies were performed at 15, 30, 45 minutes, and then hourly intervals after pancreatitis had been induced with bile. Thirty-eight dogs were used. The animals were permitted to wake up and were given water freely. No postoperative treatment was given. No attempt was made to correct the diabetic acidosis which occurred late. There were marked differences in survival time. Only those animals in which the pancreas had been removed during the first 30 minutes of their disease showed any increase in survival time over nonpancreatectomized control animals with pancreatitis. Those animals operated upon after 30 minutes, although still in good condition clinically, had mean survival times equal to nonpancreatectomized dogs with bile pancreatitis. Pancreatectomy performed on normal dogs resulted in survival times greater than any of the dogs with pancreatitis. These results (Table 1) compared closely with a similar study by Rittenbury and Egdahl.[10] Their conclusions that "A toxic substance or its precursor is formed and released during the early phase of experimental acute hemorrhagic pancreatitis in sufficient amounts to cause death irrespective of the continued presence of the gland," seem justified by our studies. This study clearly establishes the unusual nature of the pancreas as compared to other tissues undergoing necrosis such as bowel where resection of the infarcted tissue, even late in the course of the disease, is often attended by survival.[11]

2. The Early Rise in Interstitial Trypsin Activity.—In order to determine the role of trypsin in the development of pancreatitis, the interstitial edema fluid which develops rapidly after pancreatitis was first chosen for assay. This fluid was aspirated through a No. 27 gauge hypodermic needle at various intervals after pancreatitis and assayed immediately for trypsin. In nine dogs results were obtained which were similar. A representative study is shown in Fig 1. The levels of trypsinlike activity in the interstitial fluid rose rapidly, reaching maximal concentration in 45 minutes to one hour and then decreasing over the next three hours. This is of particular interest in view of the finding that removal of the pancreas after the 30-minute interval fails to ameliorate the shock syndrome.

TABLE 1.—*The Effect of Pancreatectomy in Acute Pancreatitis*

Dogs, No.	Time of Pancreatectomy, Hr	Av. Survival Time, Hr
3	Pancreactectomy alone	50
5	0-½	40
5	½-1	18
5	1-2	16
5	2-3	17
5	3-4	15
10	Pancreatitis alone	17

3. Proteolytic Activity and Vasoactive Polypeptides in Peritoneal Fluid.—To trace further the sites of proteolysis, peritoneal fluid accumulating during pancreatitis was

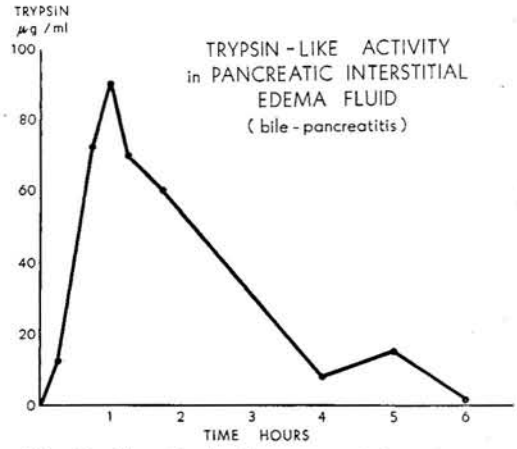

Fig 1.—Trypsin levels measured in pancreatic subcapsular edema fluid every 15 minutes after pancreatitis.

*LSD supplied by Sandoz Laboratories.

† Dibenzyline supplied by Smith, Kline & French.

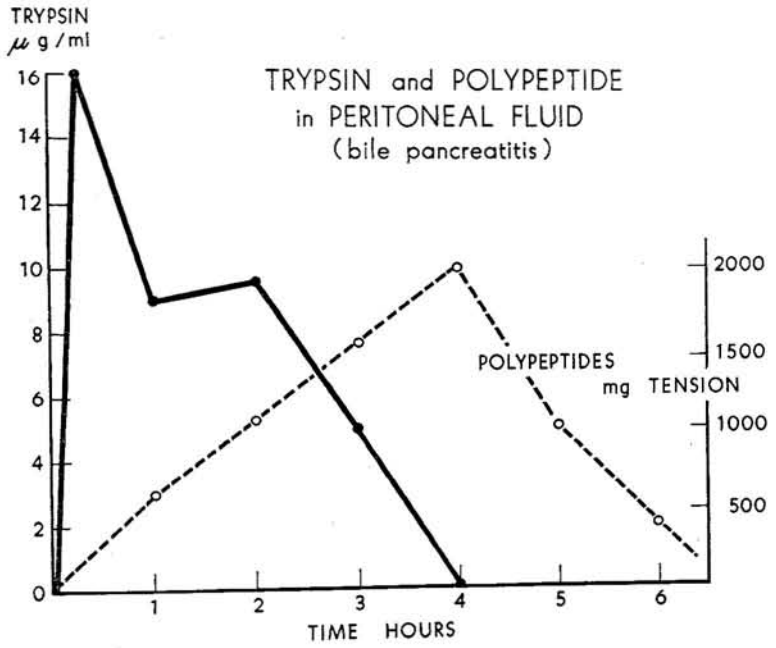

Fig 2.—Trypsin and polypeptide levels measured in peritoneal fluid during the course of pancreatitis.

next assayed for trypsinlike activity as well as polypeptides as previously defined. This fluid, which rapidly develops after induction of pancreatitis, frequently exceeds 500 cc. Again a very early rise was noted in trypsin activity. Although the concentration of trypsinlike material is much less than in the interstitial fluid, this nonetheless represents a considerable amount of trypsin diluted in a large volume of ascitic fluid. Polypeptides rose more gradually, reaching maximal levels in three to five hours. If this fluid is allowed to incubate in vitro for several hours before assay, polypeptide levels increase considerably, reaching two to three times the value shown. This confirms an enzymatic mechanism of release. A representative result is shown in Fig 2.

4. Indirect Measurement of Proteolytic Activity in Blood From the Pancreaticoduodenal Vein.—Because of the many problems inherent in measuring proteolytic activity in blood, an indirect approach was attempted. This is based on the ability of trypsin to liberate the polypeptide bradykinin from plasma α-2 globulins. These polypeptides were measured in four dogs in pancreaticoduodenal vein blood collected at five-minute intervals in siliconized and heparinized tubes after the induction of pancreatitis and allowed to incubate for 20 hours at 37 C.

Assays for vasoactive polypeptides were made. A representative result is shown in Fig 3. It will be seen that release of polypeptides is greatest from the blood collected during the first 30 minutes. It can also be reasoned that trypsin must be released during this very early period.

Although these results seem to be at odds with the previous data in which it was stated

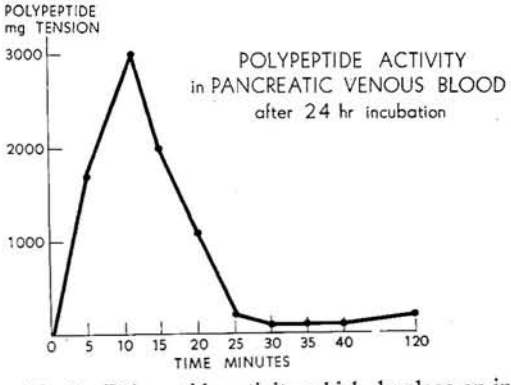

Fig 3.—Polypeptide activity which develops on in vitro incubation of pancreatic venous blood after pancreatitis (20 hr incubation). At each interval the polypeptide level which develops in the blood collected before pancreatitis is subtracted from the total vasoactivity. In this particular figure the control was 400 mg tension.

that pancreatectomy during the first 30 minutes of pancreatitis affords a significant amount of protection, many factors must be kept in mind. Firstly, release into the blood stream directly is only one avenue of trypsin escape in pancreatitis. Egdahl has shown in the case of amylase, that peritoneal and hence lymphatic absorption might be even more significant.[12] Furthermore, Thal [13] has shown in the transilluminated pancreas preparation that within 15 to 20 minutes after the injection of bile, circulation to the pancreatic acini has largely ceased. This is due to the direct effect of bile salts on red cells and capillary endothelium. Also by this time the damaged areas become very edematous, further compromising the blood supply. Thus the blood collected after 30 minutes and the lack of demonstrable proteolytic activity in it, might be the result of perfusing the less severely damaged areas of the pancreas and surrounding structures.

As a control study, since bile could conceivably escape into this blood after intraductal injection, bile and saline and bile and blood were assayed after a similar incubation. The amount of stimulating material was small when compared to that in pancreatitis.

5. Systemic Release of Vasoactive Materials During Pancreatitis.—Previous work by Rocha e Silva has already shown that when trypsin was perfused through the liver, not only were the bound stores of histamine released, but the polypeptide bradykinin as well. Accordingly in studying the overall circulatory effects of trypsin it seemed important to measure histamine, serotonin, and catecholamines as well as polypeptides. These studies were made on the ox carotid strip using specific pharmacologic blocking agents. A typical study is seen in Fig 4. Early in the course of the disease these vasoactive materials are present in low concentration, and blood pressure remains stable. A gradual rise occurs until the three- to four-hour period when histamine and polypeptides increase sharply and in parallel. This corresponds precisely with the onset of hypotension. Usually at this point the concentration of pressor amines levels off or decreases. Lightly anesthetized dogs given pancreatitis and allowed to wake up, developed extremely high vasoactive polypeptides (2,000-4,000 mg tension) terminally (15-24 hours).

6. Measurements of Vasoactive Substances in Human Pancreatitis.—While the importance of histamine may be exaggerated in the dog due to the large bound stores in the liver, preliminary studies in human acute pancreatitis reveal a similar overall pattern in the release of histamine and polypeptides. A number of clinical cases of acute pancreatitis have been studied. Polypeptides and histamine were measured. In general, the more acute the pancreatitis, the higher the polypeptide levels. Acute exacerbations of chronic alcoholic pancreatitis gave lower

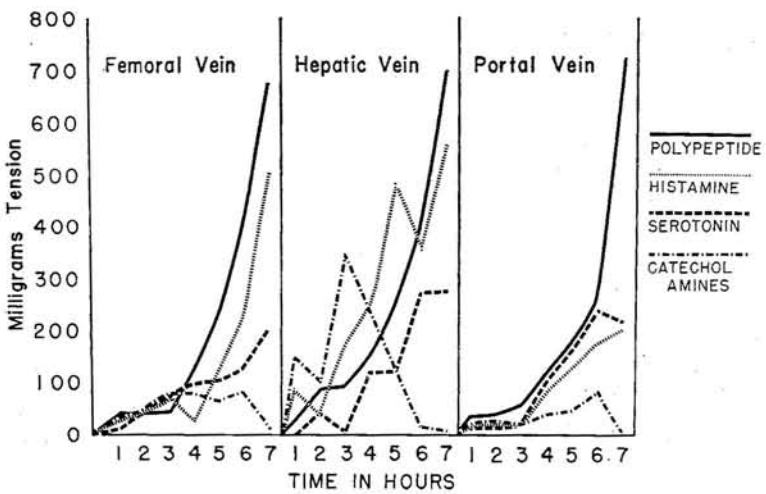

Fig 4.—Experimental pancreatitis; levels of circulating vasoactive substance were measured at hourly intervals at various sites.

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TABLE 2.—*Polypeptide Levels in Clinical Pancreatitis*

		Amylase	Polypeptides
40 M	Hemorrhagic pseudocyst	360	820
42 M	Acute pancreatitis	280	1,500
60 F	Acute pancreatitis	288	400
32 M	Chronic pancreatitis	308	300
47 M	Chronic pancreatitis	288	400
20 M	Pancreatic contusion	624	1,800
50 F	Acute pancreatitis	1,000	1,500
39 M	Severe chronic pancreatitis	192	600
29 F	Ruptured pseudocyst	638	840
31 M	Acute pancreatitis	200	660
35 F	Acute pancreatitis	200	1,440
42 M	Acute pancreatitis	406	1,200
33 M	Acute pancreatitis	538	1,200

values. Acute hemorrhagic pancreatitis resulted in polypeptide levels of 800-1,800 mg of tension which would correspond to 0.1-0.3μg of bradykinin/ml of blood. The chronic alcoholic forms of pancreatitis during exacerbations gave values of 200-800 mg of tension. The data is compared with the serum amylase in Table 2.

Comment

The present studies suggest that in experimental pancreatitis toxic agents are released early in the course of the disease. This sets in motion a chain reaction which is not influenced by removal of the pancreas.

Many approaches can be taken in the investigation of this phenomenon. In this study we have chosen to measure the release of trypsin in the pancreas and the secondary effects resulting from this enzyme.

The problem of measuring trypsin in biologic materials accurately is fraught with many difficulties. Measurement in blood presents an especially difficult problem. A complex trypsin inhibitor system exists in blood where 90% of this inhibitor is in the α-1 globulin and 10% is in the α-2 globulin.[14] The inhibitory capacity of blood has been repeatedly shown to be between 800-1,000μg/ml.[15,16] Blood, therefore, has an enormous capacity for inhibiting trypsin, and yet this enzyme has been shown to have deleterious effects when given intravenously. A partial answer to this discrepancy might be in the recently discovered trypsin-binding protein in the α-2 globulin fraction.[17,18] This protein, which binds 100μg-200μg of trypsin per milliliter of blood, protects the enzyme from naturally occurring inhibitors as well as soybean trypsin inhibitor. In this bound form trypsin is proteolytically active. A trypsin-kallikrein inhibitor (Trasylol ‡) used extensively in the treatment of pancreatitis, has been shown to inhibit this bound form of trypsin in our laboratory.

Biopsies of the pancreas taken during pancreatitis, and then homogenized, failed to demonstrate any trypsin activity, but on fractionation of the dog pancreas, a potent trypsin inhibitor was found in the supernatant fraction which completely inhibits any active trypsin present. This has been demonstrated by others as well.[19] Undoubtedly the release of inhibitor in the process of homogenization accounts for our inability to measure trypsin in extracts of pancreas.

Since we were unable to measure trypsin in pancreatic homogenates, aspirates of interstitial edema fluid were used, thus avoiding the possibility of lysing viable cells and releasing inhibitor. Early release of enzymes in experimental bile pancreatitis has also been demonstrated by Egdahl [12] who measured amylase and lipase in lymph, pancreaticoduodenal vein blood, and peripheral vein blood. The importance of peritoneal absorption through the lymphatics was stressed. The early release of trypsin is also suggested by the fact that blood in the pancreatic venous effluent has the ability to liberate polypeptides only within the first half hour after induction of experimental pancreatitis.

Recently the entire concept of tryptic pancreatitis was challenged by Beck [20] who was unable to demonstrate active trypsin in homogenates of pancreatic biopsies taken during bile pancreatitis. Here again, it appears that pancreatic trypsin inhibitor destroyed active trypsin during the process of homogenization.

The forceful intraductal injection of bile sets in motion a severe necrotizing process.

‡ Trasylol supplied by FBA Pharmaceuticals, New York, NY.

Although it is not clear whether or not bile can convert trypsinogen into trypsin and much evidence exists to the contrary, there is evidence that bile is important per se in initiating and activating this experimental form of pancreatitis. Using the synthetic substance, TAMe, a mixture of bile and inactive pancreatic juice will develop esterolytic activity.[9] Furthermore, bile has some ability to inhibit the pancreatic trypsin inhibitor.[9] Microscopic observation of the effects of bile on the pancreatic microcirculation demonstrate almost total stagnation of blood flow within a few minutes. Once activated, trypsin alone is not capable of destroying viable cells, but accompanied by the surface-active bile salts, cytolysis is likely to occur. Many other factors have been implicated in the activation of trypsin and are all probably operative to some degree. "Cytokinase," lysosomal cathepsins, not to mention ischemia produced by the volume of injected material, are all thought to play some role in the activation of trypsinogen.[21]

Once activated and liberated, trypsin is unlikely to produce its effects directly as in trypsin shock. Furthermore, it is unlikely that we are dealing with the massive amounts of trypsin used in the trypsin shock experiments. On the other hand, Rocha e Silva has shown that trypsin liberates bound stores of histamine and the hypotensive polypeptide bradykinin from blood and tissue. This appears to be important in pancreatitis where shortly after induction the capsule becomes very permeable to the contained trypsin. Peritoneal collections of this enzyme appear almost as soon as it is liberated in the pancreatic interstitium. Copious ascites then dilutes this enzyme which is involved in the liberation of a polypeptidelike material. The ascitic fluid serves as an ideal medium since as an exudate it seems to contain the α-2 globulin substrate needed for liberation of polypeptides, but does not contain much trypsin inhibitor. Trypsin probably is not absorbed from the peritoneal cavity as such but liberates the polypeptides which are then rapidly absorbed. The levels of circulating polypeptides as well as histamine correlate very closely with the hypotension.

The importance of the parallel rise in histamine must not be underestimated. Schayer[22] has recently reexamined the physiologic role of histamine and feels that an induced or newly synthesized form of histamine might be the sole mediator of the microcirculation. In any event, the release of polypeptide and histamine in human pancreatitis is now well documented and may explain the facial flush, plasma loss, and hypotension which are seen in acute necrotizing pancreatitis.

It thus appears that these very complex mechanisms can be directly related to the early minutes of this experimental form of pancreatitis. The critical nature of this phase is certainly suggested by the increased survival time with early pancreatectomy. In addition, Thal and Hollenberg[23] have shown that early pancreatectomy prevents the further rise in vasoactive polypeptides. The time lapse between this early phase and later rise in vasoactive substances demonstrates the enzymatic nature of this process since blood taken shortly after pancreatitis and allowed to incubate will parallel the in vivo generation of vasoactive polypeptides.

Summary

It is suggested that in bile pancreatitis there is a sudden release and activation of the preformed zymogen. Further protein synthesis is unlikely after the local circulatory arrest and edema induced by bile. It is also probable that this brief massive release of proteolytic enzyme constitutes the major contribution of the pancreas to the progressive deterioration of the animal. The mechanisms involved are discussed.

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Generic and Trade Names of Drugs

Pentobarbital—Nembutal.
Tromethamine—Talatrol.
Phenoxybenzamine hydrochloride—Dibenzyline.
Pyrilamine maleate—Copsamine, Neo-Antergan maleate, Paraminyl maleate, Pyra-maleate, Pyramal

maleate, Pyrilamine maleate, Stamine, Stangen maleate, Statomin maleate, Thylogin maleate.

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DISCUSSION

ON PAPERS BY DRS. KELLY, BRATCHER, AND FALOR AND DRS. KATZ, SILVERSTEIN, KOBOLD, AND THAL.

HARVEY BERNARD, MD, St. Louis: I enjoyed reading Dr. Kelly's paper, and I am sure you will, too.

However, I believe this line of investigation will be more valuable in unraveling the etiologic puzzle than in solving the the therapeutic one.

We are given good reasons to suspect that the antitrypsins must be given very early to become effective. While we have had no experience with trypsin inhibitors, we have had sufficient experience to know the difficulty in establishing a diagnosis of acute pancreatitis; in fact, in 40% of our patients dying with acute hemorrhagic pancreatitis, the diagnosis was apparent first at necropsy. I fear that in our hands, at least, the first dose of antitrypsin would be very much delayed.

A colleague, Dr. Grenier of Strasbourg, has had considerable experience with the antitrypsin drugs. The results following multiple injections of 100,000 units of Trasylol intravenously in over 140 patients have been unsatisfactory. He has injected 50,000 units daily, both intravenously and intraperitoneally, in dogs immediately after pancreatitis had been produced in the way described by Dr. Kelly. He observed a reduction of amylase and lipase in the serum and the thoracic duct lymph of the treated animals.

However, in groups which are comparable in size to those reported by Dr. Kelly, all of the dogs died except those that had been treated by injections of this material intraperitoneally about the pancreas—peripancreatic injections. As you see (slide), he has worked on the injection of other materials as well.

The evaluation of this material for the treatment of human pancreatitis requires a very carefully controlled study based on precise diagnostic criteria, for otherwise it will be impossible to tell whether suc-

cess merely occurred in the less severe forms of the disease.

In our experience, this means that laparotomy is necessary. Perhaps Dr. Grenier's technique offers a great deal in this regard.

FRASER N. GURD, MD, Montreal: I would like to discuss both of these interesting papers. Drs. A. G. Thompson and T. H. B. Haig of The Montreal General Hospital have recently published a paper not unlike that presented by Dr. Kelly, and reporting similar results. (Canad J Surg 7:97 [Jan] 1964.) Dr. Thompson and his co-workers use the method of Elliott, involving low-pressure injection of a mixture of bile and trypsin.

In addition, by measuring the release of bradykinin in pancreatitis, and using the kallikrein inhibitor Trasylol as a qualitative antidote, Dr. Thompson's group has produced evidence for the importance of bradykinin release in the production of the drops in blood pressure and in plasma volume. Their method of identifying the vasoactive material has differed from that reported by Dr. Katz and colleagues. They have used the method of Rocha e Silva to estimate the fall in plasma bradykininogen as an index of bradykinin release. Their working hypothesis is that in acute pancreatitis trypsin and kallikrein are liberated in the pancreas; kallikrein in turn activating bradykinin from the α-2-globulin fraction to produce the fall in blood pressure, vasodilatation, pain, and extravasation of plasma.

We have used Trasylol clinically in pancreatitis. We have not overcome the problem of establishing satisfactory controls, but we have been impressed by its value when used as a continuous intravenous infusion over a period of three to four days.

No side effects have been noted but its power of relieving pain may be noted in conditions other than pancreatitis. For instance, in one case of perforated ulcer, and in another of ruptured spleen, the relief of pain upon starting the Trasylol infusion was quite striking. This is a point to remember in connection with its use, as it might conceivably confuse the diagnosis.

I have enjoyed both these papers very much indeed and look forward to further work from both these sources.

ALAN P. THAL, MD, Detroit: Dr. Gurd's summary reflects our experience with the liberation of the vasoactive polypeptide.

In regard to Dr. Kelly's paper, I think the most important point he has brought out is the necessity for early inactivation of this process which, once set in motion, goes inexorably on to a fatal end.

This ties in very nicely with Dr. Katz's demonstration of the protective effect of early pancreatectomy in the first 30 minutes. This is rather a unique phenomenon. We are all aware that both in the experimental animal and in man the delayed resection of a loop of gangrenous bowel responsible for producing shock will result in rapid improvement.

On the other hand, delayed resection in pancreatitis, as Dr. Katz has shown, is unavailing. Surgeons have been well aware of this for some time and have noted the peculiarities of pancreatitis and the singular features of this disease. This is really an instance of what we are looking for.

Why is it different from these other necrotizing conditions? We think perhaps the work Dr. Katz and his colleagues, Dr. Gurd's group and that of Rocha e Silva have shown that this gland does have the unique ability to liberate agents capable of splitting vasoactive polypeptide off the large plasma globulin fragments and in addition releasing other agents, most particularly histamine. All of these substances are capable of producing the triad of pain, edema, and hypotension which so uniquely characterizes pancreatitis. The unique edema of the disease, the pain, the unusual severity of the pain, the hypotension, change in permeability, change in plasma volume and hematocrit—all these effects are well explained by these agents liberated by proteolytic enzyme.

It is interesting that the agent Trasylol, which perhaps has an unfortunate name and an indefinable chemical structure as yet, does have important actions in other areas. It is important in preventing the activation of the plasminogen-plasmin system. It is even important, as has recently been shown, in certain types of snake bite including the bite of the African adder and certain other snakes. While this may seem superficially to resemble a panacea, in all these conditions there is this common proteolytic mechanism.

As you know, snake venom often contains a proteolytic enzyme much akin to the enzymes released by the pancreas, and it might be worthwhile to consider that the pancreas may represent the venom gland of the abdomen.

HAROLD B. HALEY, MD, Chicago: In studying inflammatory processes of any type, be it the metabolic response to trauma, wound healing, or in the model we have been discussing in the last two papers, experimental pancreatitis, it is always a difficult problem to separate the differing actions of complex, poorly-defined, overlapping agents. In these cases we have been talking about proteolytic enzymes, catecholamines, tissue amines, and probably a dozen other compounds we do not recognize at the present time.

One step we have to take in carrying out studies in this area is to try to dissect out various components of this complex of factors. One substance which has been mentioned very nicely in Dr. Katz's work is the histamine release. I would like to direct a comment or two to this.

Histamine in the body as a general rule is released by discharge of the mast cells; so, one question I would ask is: Is the trypsin acting to discharge histamine from the mast cells throughout the body, or is this a local phenomenon?

TABLE 3.—*Kinin Formation and Destruction*

Production		Polypeptides		Destruction
Enzymes Dilution Glass Substrate		↓ Half-life ↑ Changes in disease		Kininase (carboxypeptidase) Chymotrypsin

The mesentery, for instance, is rich with mast cells; is this the source of the histamine that is discussed?

How important is the histamine action? From a biological experimental standpoint, I would think some information could be obtained on this question by carrying out repeated experiments in animals that have been depleted of histamine, in which mast cell discharge was carried out first by administrating 48/80 or ovomucoid or other substances that will do this, and then see if the same type of reaction is obtained to the experimental pancreatitis.

It could then be approached from the other standpoint of administering to the animals antiserotonin, antihistamine compounds such as cyproheptadine which cuts down the action of these compounds, and therefore we could find whether the physiological effects were from these or from the catecholamines or some of the other factors in this complex of many different biological agents that are released in such experiments.

THOMAS R. KELLY, MD, Akron, Ohio: I wish to thank Drs. Bernard, Gurd, and Thal for their remarks. I think what Dr. Bernard mentioned is the general opinion now. Certainly most of us have been able to show that Trasylol is very effective in dogs but not as effective in humans, and mostly because of the time element.

I cannot explain why Dr. Bernard's one reference did not get good results in the dog, using Trasylol intravenously, except that there are several ways to produce acute hemorrhagic pancreatitis, and each way gives a different mortality in the control group; so this is a very difficult thing to keep stable.

We feel Trasylol is very effective in inhibiting proteolytic enzymes and that our study provides a theoretical basis for its use in man. The main indications would be therapeutically in acute pancreatitis and prophylactically in the prevention of postoperative pancreatitis. I think this could be kept in mind, for instance, after doing a difficult gastric resection when one has to manipulate the pancreas quite extensively. This is a good opportunity to start your treatment with Trasylol early.

The best results, of course, will be achieved in those patients in whom the drug has been given prophylactically or very early in the course of the disease. This would not of necessity preclude a beneficial effect to the patient in which the pathological process of pancreatitis is well under way, since high dosages of the inhibitor may well counteract the necrotizing activity of the proteolytic enzymes before irreversible changes ensue.

This point is substantiated by an incident that happened to a medical colleague treating a Gypsy proven by operation to have acute hemorrhagic pancreatitis. It was made clear to the surgeon by

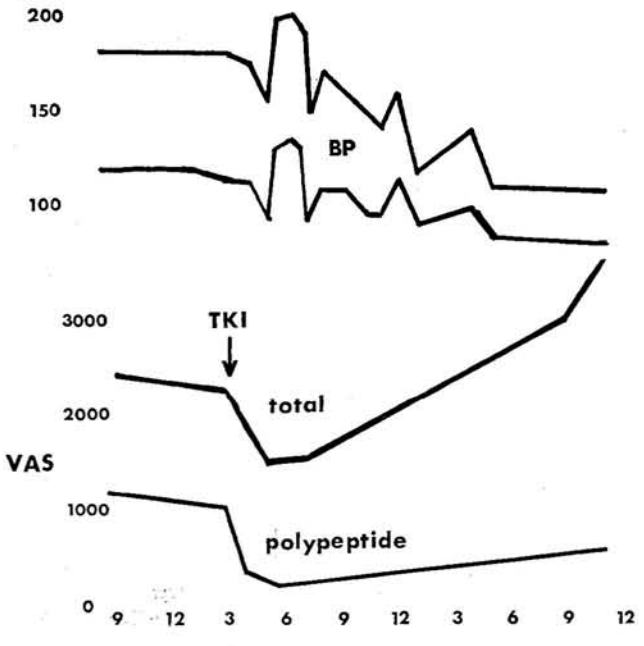

Fig 5.—Example of patient with laparotomy confirmed acute hemorrhagic pancreatitis.

the queen Gypsy that, should the patient die, so might the surgeon. The surgeon, desiring to remove himself from such a predicament, willingly used some of the Trasylol used in our study. Some time later, I asked my colleague about the condition of his patient, and he stated that the patient was home in good health and that he, the surgeon, likewise was enjoying good health.

W. KATZ, MD, Detroit: I would like to thank the discussers for their provocative comments. I have two slides that might clear up some of the points they have raised.

This (Table 3) is a concept we have evolved about the formation and destruction of polypeptides which might explain the action of Trasylol in this process. On the one hand, we have production of these materials by enzymes, dilution, glass contact, and substrate availability. On the other hand, we have destruction of these polypeptides by bradykininase which most likely is carboxypeptidase, and chymotrypsin. In pancreatitis both trypsin and the kininase are increased but the polypeptides are being produced at a rate faster than the kininase can inactivate them.

Here (Fig 5) is an example of a patient with laparotomy confirmed acute hemorrhagic pancreatitis. The two lines at the bottom of the slide represent total vasoactivity which encompasses histamine and pressor amines as well, and polypeptides. The "TKI" represents trypsin-kallikreinin inhibitor, Trasylol. After giving 200,000 units of Trasylol intravenously, there is a very dramatic drop in total vasoactivity and polypeptide levels. Within six hours or less, there is a rapid return of the total vasoactivity and a more gradual increase in the polypeptides. Thus by inhibiting the producing enzyme, trypsin, the kininase can rapidly destroy the polypeptides.

Not shown on this slide is the increase in histamine which is much more rapid than polypeptide. Also seen is a concomitant increase in blood pressure after injection of the trypsin-kallikreinin inhibitor.

I believe this slide shows the importance of giving much more frequent and larger doses of Trasylol than are currently used.

Our results, using Trasylol clinically, have been rather disappointing. This is primarily due to the difficulty in diagnosing pancreatitis accurately. At the present time we are trying to get around this by running a double-blind study.

Pancreatoduodenectomy

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Since 1935 when Whipple et al [1] described a procedure for resecting the head of the pancreas, discussion has continued concerning the indications for this operation. That this question remains unresolved is indicated by the differences of opinion expressed by competent surgeons in the current medical literature. At one extreme is the pessimistic view that pancreatoduodenectomy is rarely if ever indicated for treatment of carcinoma of the head of the pancreas.[2] In contrast is the optimistic view suggesting that the operation should be used to treat carcinoma of the head of the pancreas as well as carcinoma of the ampulla of Vater.[3] High operative mortality and postoperative morbidity rates are the principal reasons for the continued caution concerning the indication for this operation. Reported operative mortality rates vary between 6.5%[4] and 47%.[5] To justify an operative risk of this magnitude it is necessary to have evidence that pancreatoduodenectomy, when compared with lesser operations or no operation, relieves the distressing symptoms of biliary tract obstruction and results in objective improvement and longer survival. This is the report of a clinical investigation in which the operative mortality and morbidity and postoperative survival of patients subjected to pancreatoduodenectomy were compared with the similar findings in patients treated by biliary bypass procedures and the findings in a third group of patients who were only explored. The history, preoperative findings, and operative and postoperative

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