VASCULAR FACTORS IN THE PATHOGENESIS OF ACUTE HAEMORRHAGIC PANCREATITIS

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by

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ACUTE PANCREATITIS IS the acute inflammatory response of pancreatic tissue to different types of injury. The pathological changes which may be seen in the gland range from minimal oedema to extensive haemorrhagic infarction (Popper *et al.* 1948; Thal *et al.* 1957). This disease was not recognized during John Hunter's lifetime but, in his *Treatise on the Blood, Inflammation and Gun Shot Wounds*, Hunter gave an accurate description of many of the vascular changes which occur during inflammation. It is now known that many of the changes he described occur in the pancreas during an attack of pancreatitis. Despite numerous and extensive studies, many aspects of this disease are ill-understood and treatment therefore is of an empirical nature and often unsatisfactory.

The overall mortality of acute pancreatitis is still high and has varied between 13.8 per cent (Kirby *et al.* 1955) and 33.3 per cent (Paxton and Payne, 1948). Unless it is complicated by haemorrhagic necrosis, oedematous pancreatitis is associated with a good prognosis (Elman, 1946) and the mortality is approximately 3.4 per cent (Albo *et al.* 1963). Haemorrhagic pancreatitis develops in 20–25 per cent of all cases (Thal *et al.* 1957) and is associated with a considerable increase in mortality, which varies between 33 per cent (Kirby *et al.* 1955) and 78 per cent (Sinclair, 1959). In view of this formidable mortality, a clearer understanding of the pathogenesis must be obtained if the prognosis is to be improved.

This lecture is concerned only with the pathophysiological changes in the gland, and the many aetiological factors which are known to play a part in the initiation of an attack of acute pancreatitis will not be discussed.

The relationship between oedematous and haemorrhagic pancreatitis has been studied extensively and it is generally accepted that the latter is usually preceded by an oedematous phase. Quick (1932) described a patient with oedematous pancreatitis, confirmed at operation, who died a few days later from extensive pancreatic necrosis. Popper (1946) made similar clinical observations and, in addition, he and his co-workers described canine experiments in which exocrine hypersecretion was induced after ligation of the pancreatic ducts. They found that pan-

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creatic oedema developed invariably but, if the arterial blood supply to the gland was interrupted as well, haemorrhagic pancreatitis developed in all cases (Popper *et al.* 1948). Adams and Musselman (1953) later demonstrated that if venous obstruction occurred in the presence of pancreatic oedema, haemorrhagic infarction similarly resulted. Anderson (1961, 1963) has suggested that free trypsin released into the interstitium of the gland during an attack of pancreatitis reacts with the haemoglobin from extravasated erythrocytes to form a vasotoxic substance. This substance is related to haemochromogen (Nemir *et al.* 1959) and results in further vascular damage with subsequent thrombosis and secondary parenchymal necrosis (Anderson, 1961).

A. QUALITATIVE STUDIES

A series of experiments were devised to investigate the hypothesis that, during an attack of acute pancreatitis, a decrease in blood flow in the pancreatic microcirculation is the significant factor which results in the development of pancreatic necrosis. A potentially lethal haemorrhagic pancreatitis was induced in dogs. The animals were then treated with different therapeutic agents which were given with the aim of preventing a reduction in blood flow taking place in the microcirculation.

Method of inducing pancreatitis

A potentially lethal haemorrhagic pancreatitis was induced in each dog by a modification of the method described by Anderson (1961). This method is based on the fact that a potent vasotoxic substance is formed when trypsin is incubated with blood under sterile conditions.

The animals were anaesthetized with intravenous pentobarbitone (25 mg./kg.) and aseptic operative techniques were used. The main and lesser pancreatic ducts were identified at operation and the latter ligated, special care being taken to avoid damage to the common bile duct, which is in close proximity. A duodenotomy was done opposite the opening of the main duct, which was cannulated with polyethylene tubing and injected with 0.75 ml./kg. of trypsin digested blood under a pressure of 140 mm. Hg. The tubing was then removed and the duct ligated. The duodenotomy and abdomen were then closed.

Preparation of trypsin digested blood

Twenty-four hours prior to induction of pancreatitis, 10 ml. of autologous blood was removed from the femoral vein under sterile conditions. The blood was added immediately to an equal volume of sterile normal saline, containing 25,000 units of trypsin* per ml., in a sterile container and incubated at 37° C. for 24 hours.

Evaluation of the pathological changes

An autopsy was done on each animal as soon after death as possible or, if survival occurred, at the chosen arbitrary time of sacrifice at 72 hours. Haemorrhagic pancreatitis was considered to be present if parenchymal

* ' Tryptar ', a crystallized preparation of trypsin, Armour Pharm. Co.

necrosis was found. An independent evaluation of the gross and histological changes was made and graded as severe, moderate or mild haemorrhagic or oedematous pancreatitis.

The effect of low molecular weight dextran,* clinical dextran and plasma

Low molecular weight dextran has been shown to improve blood flow in the microcirculation in conditions in which it is known to be impaired (Bergentz *et al.* 1961). Its mode of action is complex and includes not only a direct effect on blood flow by a specific erythrocyte disaggregating effect (Rothman *et al.* 1957), but also indirect effects by virtue of antithrombotic (Chopra *et al.* 1967) and hyperoncotic properties (Ahnefeld *et al.* 1965). Initial experiments demonstrated that administration of this substance prevented haemorrhagic pancreatitis from developing and, therefore, in an attempt to elucidate the relative importance of these three factors, further groups of animals were treated with clinical dextran and with plasma.

Induction of pancreatitis and post-operative treatment

Pancreatitis was induced in all the animals in this series by injecting trypsin digested blood into the pancreatic duct. Each animal received an intravenous infusion of dextrose/saline (60 ml./kg.) during and immediately after operation. This was repeated every 24 hours until death or sacrifice at 72 hours. In addition, at the end of the operation, the control group received 10 ml./kg. normal saline, the plasma group 10 ml./kg. plasma, the clinical dextran group 1 gm./kg. of 6 per cent clinical dextran in normal saline, and the low molecular weight dextran group 1 gm./kg. of 10 per cent low molecular weight dextran in normal saline. This treatment was repeated every eight hours until the time of death or sacrifice.

Results: These are summarized in Table I.

TABLE I

ACUTE PANCREATITIS

Effect of Immediate Treatment

Treatment	*No.*	*Mortality*	*Haemorrhagic pancreatitis*			*Oedematous pancreatitis*		
			+++	++	+	+++	++	+
Normal saline	25	25	18	5	2	—	—	—
Plasma	13	4	6	1	2	2	2	—
Dextran	10	2	2	2	1	1	2	2
L.M.W. dextran ..	12	—	—	—	—	2	2	8

Control group

The control dogs treated with normal saline all died in less than 72 hours. The mean survival time was only 28 hours. These dogs had marked vomiting and prostration post-operatively. Haemorrhagic pancreatitis, usually severe in degree, was present in all cases. Oedematous pancreatitis was not found in this group. The microscopic appearances were characterized by extensive areas of parenchymal

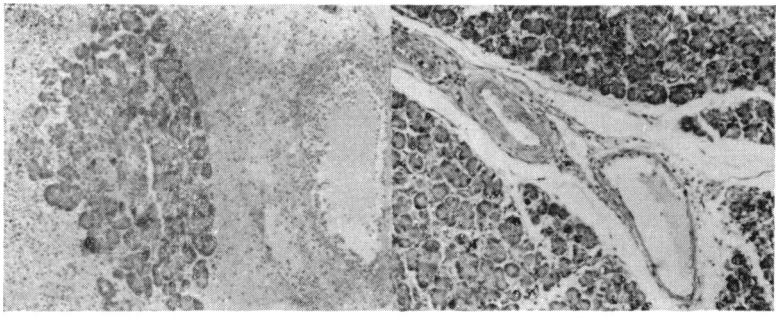
* ' Rheomacrodex ', Pharmacia Ltd.

necrosis with severe inflammatory changes in the arteries and veins (Fig. 1*a*). Capillary congestion and hyaline necrosis were common findings throughout the gland.

Plasma group

The mortality was reduced in this group to 30 per cent. The four fatalities had a similar post-operative course to those in the control group treated with normal saline. The other animals pursued a variable course, but overall the symptoms and signs tended to be severe. In 69 per cent (9) of the animals, haemorrhagic pancreatitis was present and usually severe, whilst in the remaining 31 per cent (4) there was severe or moderate oedematous pancreatitis.

Comment: A considerable reduction in the mortality from acute haemorrhagic pancreatitis can be achieved if the severe hypovolaemia, with which it is associated, is combated by appropriate therapy. The



(*a*) (*b*)

Fig. 1. (*a*) Control group treated with normal saline. Extensive parenchymal necrosis with severe interstitial oedema and extensive leucocytic infiltration and haemorrhage. Severe phlebitis with thrombosis and margination of leucocytes. (Haematoxylin and eosin × 50.) (*b*) Low molecular weight dextran group. Mild interstitial oedema with no evidence of parenchymal or vascular necrosis. (Haematoxylin and eosin × 50.)

morbidity, however, judged by the development of haemorrhagic pancreatitis, was only slightly reduced.

Clinical dextran group

Treatment with clinical dextran resulted in a significant reduction of the mortality to 20 per cent. The post-operative course of these animals was most variable. The majority had severe symptoms, however, and in 50 per cent some degree of haemorrhagic pancreatitis was found at autopsy.

Comment: The mortality was considerably reduced by treatment with clinical dextran, which has both the antithrombotic and plasma-expanding properties of low molecular weight dextran but not the specific blood flow improving property (Borgström *et al.* 1959). The results were not completely satisfactory, however, since some degree of haemorrhagic pancreatitis was present in 50 per cent of the animals.

Low molecular weight dextran group

There were no deaths in this group. In contrast with the other groups, haemorrhagic pancreatitis did not develop in these animals, who had only minimal symptoms post-operatively and appeared normal on the second and third day. At autopsy, the pancreas either appeared normal or, as in the majority of cases in this group, showed only minimal oedema. Minimal interstitial oedema and leucocytic infiltration were the only histological findings and the blood vessels appeared normal (Fig. 1*b*).

Comment: In conclusion, it is apparent that there exists a spectrum of changes which range from the most severe in the control group, followed by less severe changes in the plasma group, moderate changes in the clinical dextran group, and the least severe changes of all in the low molecular weight dextran group. The success of the latter substance is attributed to a number of factors, but it is suggested that it had a better therapeutic action than clinical dextran because of its specific erythrocytic disaggregating effect. This prevented both the development of stasis and the formation of haemagglutination thrombi in the pancreatic microcirculation. A satisfactory blood flow was maintained and haemorrhagic pancreatitis failed to develop.

The effect of Thrombolysin* and sympathectomy

Induction of pancreatitis and post-operative treatment

Pancreatitis was induced in all the animals in this series by injecting trypsin digested blood into the pancreatic duct. Each animal received an intravenous infusion of dextrose/saline (60 ml./kg.) during and immediately after operation as well as every 24 hours afterwards.

Thrombolysin group

Thrombolysin is a preparation of human plasmin (Pechet, 1965) and has a powerful fibrinolytic action. In this group, the animals received 50,000 units of Thrombolysin, which was given in the same volume of fluid as that given to the control group. Therapy was initiated immediately after pancreatitis had been induced and repeated every eight hours subsequently.

Results: In contrast with the control dogs treated with normal saline alone, these animals had only mild symptoms post-operatively and appeared normal at the time of sacrifice. One dog died at 36 hours from post-operative pneumonia. Haemorrhagic pancreatitis was not found at autopsy and the oedematous pancreatitis was of a mild degree (Table II).

TABLE II

ACUTE PANCREATITIS

Effect of Immediate Treatment

Treatment	*No.*	*Mortality*	*Haemorrhagic pancreatitis*			*Oedematous pancreatitis*		
			+++	++	+	+++	++	+
Normal saline	25	25	18	5	2	—	—	—
Thrombolysin	10	1	—	—	—	1	4	5
Sympathectomy ..	8	—	—	—	—	1	2	5

* Merck, Sharpe and Dohme Ltd.

Sympathectomy group

Sympathetic denervation of the pancreas had been done, 5–7 days prior to induction of pancreatitis, by a modification of the method described by Berger and Lium (1960) for sympathetic denervation of the abdominal viscera. This operation resulted in a post-ganglionic sympathetic denervation of the gland. These animals received exactly the same volume and type of fluid post-operatively as the control animals.

Results: These animals had only mild symptoms post-operatively. Haemorrhagic pancreatitis did not develop and none died. The oedematous pancreatitis was only of a mild degree in the majority of cases.

Comment: The dramatic difference in the results from the control dogs must be attributed to the effect of Thrombolysin and sympathectomy, since all animals received exactly the same volume and type of fluid post-operatively. It seems reasonable to conclude that the antithrombotic action of Thrombolysin and the vasodilator effect of sympathectomy each prevented the development of stasis and the formation of haem-agglutination thrombi in the pancreatic microcirculation. In consequence, haemorrhagic pancreatitis failed to develop because a satisfactory blood flow in the organ was maintained.

B. QUANTITATIVE STUDIES

The qualitative studies suggest strongly that changes in blood flow in the pancreatic microcirculation are of crucial importance in determining the outcome of an attack of acute pancreatitis. In order to assess more adequately this concept and new approach in the treatment of this disease, quantitative information on the haemodynamic changes which take place were considered to be desirable.

Accurate measurements of pancreatic blood flow have, until recently, been difficult to obtain since the organ is rather inaccessible and has a complicated blood supply. The indicator fractionation technique for measuring blood flow has circumvented these difficulties to some extent (Sapirstein, 1958; Delaney and Custer, 1965).

This method is based on the assumption that, shortly after an intravenous injection of a substance such as radio-active potassium or rubidium which permeates cell membranes freely, the fraction contained in an organ is equal to the fraction of the cardiac output perfusing that organ. A measurement of the fraction contained in the pancreas, therefore, enables the organ blood flow to be calculated if the cardiac output also is known. Since it is known that a small amount of isotope recirculates (Sapirstein, 1958), the validity of this method has been investigated in some detail. It was demonstrated that radio-active rubidium behaves as the almost ideal tracer and the fraction of this isotope which is present in the gastro-intestinal organs within one to two minutes of injection is equal to the fraction of the cardiac output perfusing them (Delaney and Custer, 1965). Similar validation studies were done on dogs with oedematous pancreatitis and identical results were obtained. This

method has been shown to be accurate to within ±7 per cent, a figure which compares most favourably with the results obtained by alternative methods for measuring blood flow.

Material and methods

A total of 53 healthy mongrel dogs was used. Each dog was fasted for 24 hours prior to study. General anaesthesia was induced and maintained with intravenous sodium pentobarbitone. Blood flow studies were done on the following groups of animals:

Group 1 (19 dogs): Normal.

Group 2 (6 dogs): Sham operation. These animals were subjected to a laparotomy but pancreatitis was not induced.

Group 3 (12 dogs): Oedematous pancreatitis was induced by the injection of 0.25 ml./kg. of autologous bile into the main pancreatic duct at a pressure of 140 mm. Hg.

Group 4 (10 dogs): Haemorrhagic pancreatitis was induced by the

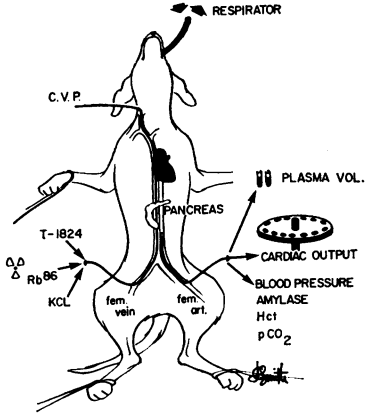


Fig. 2. Diagrammatic representation of the experimental preparation.

injection of 0.25 ml./kg. of a 1 : 1 mixture of autologous bile and trypsin into the main pancreatitic duct at a pressure of 140 mm. Hg.

In groups 2, 3 and 4, each animal was given during the operation 60 ml./kg. of dextrose/saline intravenously, followed by 10 ml./kg. normal saline eight-hourly.

Group 5 (6 dogs): Haemorrhagic pancreatitis was induced by injection of a bile-trypsin mixture in the same manner as in group 4. During operation 60 ml./kg. of dextrose/saline was given. Post-operatively, however, each animal received intravenously 1 gm./kg. of low molecular weight dextran eight-hourly, instead of normal saline.

Pancreatic blood flow was measured using radio-active rubidium (Rb^{86}) and the indicator fractionation technique, and in groups 2, 3, 4 and 5 it was determined 20 hours after pancreatitis had been induced. Immediately prior to determination, the following additional data was

collected from each dog: haematocrit, plasma volume (Evans blue dye dilution method), systemic arterial blood pressure, serum amylase (modified Sömogyi method) and the arterial pH, pO_2 and pCO_2. The rate of pulmonary ventilation was adjusted to keep the arterial pO_2 and pCO_2 within normal limits prior to determination of the cardiac output (Fig. 2).

In 12 normal and in 6 dogs with haemorrhagic pancreatitis, blood flow to the other gastro-intestinal organs was determined also.

The standard student ' t ' test for independent variates was used in the statistical evaluation of the results.

Results

Haematocrit and plasma volume: There was no significant difference between groups 1, 2, 3 and 5. In haemorrhagic pancreatitis, however, the haematocrit was increased to 56 per cent and the plasma volume reduced to 29 ml./kg.; both changes were significant ($p=<0.05$).

Mean arterial blood pressure and cardiac output: There was no significant difference between groups 1, 2, 3 and 5, but in group 4 the blood pressure and cardiac output were decreased significantly to 99 mm. Hg and to 100 ml./min./kg. respectively ($p=<0.05$).

Pancreatic weight: This was increased significantly in groups 2, 3, 4 and 5 ($p=<0.05$) and the greatest increase was found in oedematous pancreatitis.

Serum amylase: The level was unchanged after sham operation ($p=>0.05$) but significantly high levels were found in groups 3, 4 and 5 ($p=<0.05$). The greatest increase was found in haemorrhagic pancreatitis.

Total pancreatic blood flow: There was no difference in blood flow between group 1 (normal) and group 2 (sham operation). In oedematous pancreatitis, however, pancreatic blood flow was elevated significantly to 23.7 ml./min. ($p=<0.05$), which was in contrast with group 4 (haemorrhagic pancreatitis) in which the blood flow was reduced significantly below normal to 7.1 ml./min. ($p=<0.05$). In comparison with the animals in groups 1, 2 and 4, the dogs with a potentially lethal haemorrhagic pancreatitis who were treated with low molecular weight dextran (group 5) had a pancreatic blood flow which was increased significantly to 37.8 ml./min. ($p=<0.05$).

If the pancreatic blood flow is expressed as a percentage of the cardiac output perfusing the gland, the changes are similar with the exception that the percentage of the cardiac output perfusing the gland in haemorrhagic pancreatitis is reduced from the normal of 0.62 per cent to 0.39 per cent (Table III).

Pancreatic perfusion rate: There was no change from normal in the sham operated group. In oedematous pancreatitis, despite the increase in total pancreatic blood flow, the perfusion rate was significantly decreased to 43.6 ml./min./100 gm. ($p=<0.05$). A further significant decrease to very low levels was found in haemorrhagic pancreatitis (18.2 ml./min./100 gm., $p=<0.05$). In contrast, in the dogs with a

TABLE III

MEAN VALUES FOR CARDIAC OUTPUT AND PANCREATIC BLOOD FLOW IN THE FIVE EXPERIMENTAL GROUPS

Group No.	*Type of preparation*	*No. of Dogs*	*Cardiac output (ml./min./kg.)*	*Pancreatic blood flow (ml./min.)*	*(ml./min./100 gm.)*	*(per cent of cardiac output)*
1	Normal control	19	158	14.3	63.2	0.62
2	Sham operated control	6	173	17.8	54.5	0.64
3	Oedematous pancreatitis	12	168	23.7	43.6	1.01
4	Haemorrhagic pancreatitis	10	100	7.1	18.2	0.39
5	Haemorrhagic pancreatitis treated with L.M.W. dextran	6	193	37.8	70.4	1.12

potentially lethal haemorrhagic pancreatitis who were treated with low molecular weight dextran, the perfusion rate remained within the normal range.

Gastro-intestinal blood flow in haemorrhagic pancreatitis: The blood flow to the stomach, pancreas, duodenum, jejunum, ileum and colon expressed as a percentage of the normal flow in haemorrhagic pancreatitis is summarized in Table IV. Haemorrhagic pancreatitis was associated with a reduction in blood flow to all organs, but the reduction in perfusion rate in the pancreas to 23 per cent of normal was significantly greater than in the other organs ($p=<0.05$). The reduction in total blood flow to the pancreas, however, was only marginally reduced when compared with the reduction in flow to the small intestine.

TABLE IV

GASTRO-INTESTINAL BLOOD FLOW IN HAEMORRHAGIC PANCREATITIS EXPRESSED AS A PERCENTAGE OF THE NORMAL FLOW

Organ	*ml./min.*	*ml./min./100 gm.*	*per cent of cardiac output*
Stomach	71.1	50.5	90.0
Duodenum	41.3	36.0	63.0
Pancreas	37.6	23.0	50.8
Jejunum and ileum	42.4	35.2	67.9
Colon	40.4	34.7	62.7

Comment

Sham operation did not alter significantly pancreatic blood flow. Therefore the changes found in association with pancreatitis were not due to the effect of laparotomy but to the disease processes taking place within the gland.

In oedematous pancreatitis, although the total pancreatic blood flow was increased, the perfusion of pancreatic tissue was reduced to a significant extent below normal. Gross changes in capillary permeability with loss of fluid into the interstitium accounted for the increased pancreatic weight and this accounted to a great extent for the decrease in perfusion rate. The increase in total blood flow was presumably a

manifestation of the local vascular dilatation in response to inflammation and may have been due to the release of bradykinin (Ryan *et. al.* 1965), trypsin (Anderson and Bergan, 1962), trypsinogen (Anderson *et al.* 1967) or histamine (Bernard *et al.* 1958). The perfusion rate was reduced to 69 per cent of normal. Changes in perfusion rate have more significance than changes in total blood flow, since they give an indication of the volume of blood available for the metabolic needs of the cell. In oedematous pancreatitis, therefore, although the blood supply was slightly compromised, this was not to a critical level since the disease in all other respects was of a benign nature. There was no marked systemic disturbance and the haematocrit, blood pressure, plasma volume and cardiac output remained normal.

It is reasonable to postulate that haemorrhagic pancreatitis would develop if the blood supply became more severely compromised. Several mechanisms may be involved and include any or all of the following:

(*a*) sludging of erythrocytes in the capillaries and the formation of haemagglutination thrombi (Anderson, 1963);

(*b*) the release of cathepsins from damaged pancreatic cells with the activation of proelastase (Geokas, 1966);

(*c*) the release of other intracellular enzymes (Reid *et al.* 1958; Weissmann, 1965);

(*d*) the formation of a vasotoxic substance by the interaction of trypsin and blood (Anderson, 1961).

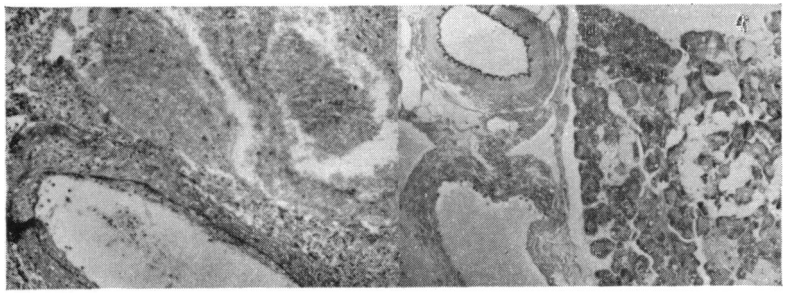
All these factors may lead to severe damage to the vascular endothelium with thrombosis and the development of haemorrhagic pancreatitis.

This series of events was reflected in the measurements of pancreatic blood flow in haemorrhagic pancreatitis. The total blood flow was reduced significantly to 29 per cent below normal. This was not due entirely to a reduction in cardiac output since there was a reduction in the percentage of the actual cardiac output perfusing the gland from 0.62 per cent to 0.39 per cent. This suggested the presence of an intrapancreatic vascular abnormality. Presumably the perfusion rate was reduced to below a critical level. This resulted in tissue anoxia and acidosis. This in turn resulted in vasoconstriction, a further increase in tissue anoxia and the development of intravascular coagulation (Hardaway *et al.* 1966). A vicious circle was created, which ultimately ended in necrosis and cellular death, if it was not interrupted before it became irreversible.

Haemorrhagic necrosis should not develop if this reduction in blood flow could be prevented, and it is presumed that the beneficial effects of treatment with low molecular weight dextran can be attributed to this. It is well documented that low molecular weight dextran will prevent or improve tissue perfusion in conditions in which it is impaired (Gelin and Ingelman, 1961), and in the animals in whom a potentially lethal haemorrhagic pancreatitis had been induced, but who were treated with low molecular weight dextran instead of normal saline, there was no

reduction in total pancreatic blood flow. In addition, the perfusion rate remained slightly above normal. Tissue anoxia, therefore, did not occur and haemorrhagic pancreatitis did not develop.

In haemorrhagic pancreatitis, the raised haematocrit and reduced plasma volume reflected a marked hypovolaemia and it has been demonstrated that in dogs this results in a reflex splanchnic vasoconstriction (Wiggers, 1950). This may account for a proportion of the reduced pancreatic blood flow and, to determine the importance of this factor, the blood flow to the stomach, small and large intestine in both normal dogs and in dogs with haemorrhagic pancreatitis was measured. In haemorrhagic pancreatitis, the blood flow to all the gastro-intestinal organs was decreased and this reduction was particularly marked in the pancreas. The reduction in total pancreatic blood flow was only marginally reduced when compared with the reduction to the duodenum



(*a*) (*b*)

Fig. 3. (*a*) Fatal acute haemorrhagic pancreatitis in man. Extensive parenchymal necrosis with severe vasculitis. Complete disruption of the vein associated with elastolysis and thrombosis. (Verhoeff's elastin stain × 50.) (*b*) Trypsin digested blood pancreatitis of three hours' duration. Moderate interstitial oedema and parenchymal necrosis. Margination of leucocytes in the vein with dissolution of the elastic tissue in the wall. Loss of the external elastic lamina in the artery but preservation of the internal elastic lamina. (Verhoeff's elastin stain × 50.)

and remainder of the small intestine. Conversely, the reduction in perfusion rate in the pancreas to 23 per cent of normal was significant when compared with the reduction in the small intestine. In conclusion, the reduction in pancreatic blood flow in haemorrhagic pancreatitis cannot be explained solely on the basis of reflex splanchnic vasoconstriction, and a specific intrapancreatic vascular lesion appears to be an additional factor.

C. BIOCHEMICAL STUDIES

It has now been established that vascular factors are important in the pathogenesis of haemorrhagic pancreatitis, but a number of questions remain to be answered. It seems likely that there is an underlying biochemical mechanism. This may be due to the abnormal activity of enzymes although the nature of these is not yet clear.

A clue may be found in the histological appearance of the haemorrhagic

necrotic gland. A characteristic feature is the presence of extensive elastolysis affecting the blood vessels (Fig. 3*a*). This affects the veins predominantly, but the arteries are also involved to a lesser degree. These lesions are found frequently both in animals and in man (Thal *et al.* 1957). It has always been assumed that these changes were due to the inappropriate release of active exocrine enzymes. It is well established that proteases and other enzymes are present in high concentration in the zymogen granules (Hokin, 1956) and also that powerful hydrolytic enzymes are present in the lysozymes (Weissman, 1965). Many enzymes have been incriminated in the pathogenesis of acute haemorrhagic pancreatitis and these include trypsin (Keith *et al.* 1958), elastase (Geokas, 1966), and intracellular enzymes (Reid *et al.* 1958).

It has been demonstrated that carefully purified trypsin is devoid of elastolytic activity (Pepler and Brandt, 1954), and therefore activation of trypsinogen cannot be directly responsible for the elastolysis which takes place. It has been demonstrated also that proelastase, the precursor of elastase, is present in the pancreas in moderate concentrations both in the dog (Geokas, 1966) and in man (Geokas *et al.* 1968). The rôle of this enzyme has been studied recently and it is the only enzyme in the dog which has been shown to have the ability to digest elastin.

In order to clarify the rôle of proelastase and trypsinogen in the pathogenesis of trypsin digested blood induced haemorrhagic pancreatitis, further studies were done.

Materials and methods

Each animal was fasted for 24 hours and anaesthetized with sodium pentobarbitone (25 mg./kg.). The main pancreatic duct was cannulated with a fine polyethylene tube which was passed 6–8 cm. into the tail of the gland, via a small duodenotomy. Acute pancreatitis was induced by injecting 0.75 ml./kg. of trypsin digested blood under a pressure of 250 mm. Hg. The head of the gland was not injected by using this technique and therefore it could be used as a control (Geokas, 1966). Three hours later the animals were killed and tissue from the head, body and tail of the gland was removed for enzymatic and histological studies.

Determination of enzymatic activity

Elastase was estimated by a colorimetric method which involved the measurement of the dye orcein, released from an elastin-orcein substrate by the action of elastase (Geokas, 1966).

Trypsin was estimated also by a colorimetric method, which involved the measurement of tyrosine released from a haemoglobin substrate by the action of trypsin (Beck *et al.* 1962).

Results

Elastase study

There was no significant elastolytic activity in the tail (Table V). Furthermore, although the concentration of proelastase in the tail was

TABLE V

FREE ELASTASE AND PROELASTASE IN TRYPSIN DIGESTED BLOOD INDUCED PANCREATITIS OF THREE HOURS' DURATION

(*Six dogs*) (*units/gm. dry tissue*)

	Head	*Body*	*Tail*	*p*
Free elastase	7.4	12.3	10.1	—
Proelastase	184.3	169.0	146.9	—

— No significant difference in head *vs.* tail ($p = > 0.05$)

reduced, the reduction was not significant ($p = > 0.05$). Histological examination of the tail showed abundant evidence of haemorrhagic pancreatitis including elastolysis (Fig. 3*b*).

Measurement of the elastolytic activity of trypsin digested blood showed negligible activity.

Comment: The intraductal injection of trypsin digested blood resulted in elastolysis of the elastic fibres in the vessel walls and this was especially prominent in the veins. Since proelastase in the affected portion of the pancreas was not activated to any significant extent, it must be concluded that trypsin digested blood had an inherent specific elastolytic activity, although it was not possible to demonstrate this biochemically. Imperfections in methodology may be the reason, since in the presence of blood or blood products the estimation of elastase has been shown to be inaccurate because of the presence of potent inhibitors (Hall, 1961).

Pancreatitis, induced by the intraductal injection of either bile or trypsin, has been demonstrated to be associated with activation of proelastase (Geokas, 1966) and low values have been recorded also in acute pancreatitis in man (Geokas *et al.* 1968). It is of interest in this context that a marked decrease in the coagulation of the blood occurs after the addition of elastase (Hall and Wilkinson, 1963) and this finding may explain the hypercoagulation state of the blood which may be found in association with acute pancreatitis (Shinowara *et al.* 1963).

Trypsin study

There was neither a significant increase in free tryptic activity nor a significant decrease in trypsinogen concentration in the tail (Table VI).

Comment: Once more it was impossible to demonstrate activation of an important enzyme in trypsin digested blood induced pancreatitis. It is conceivable again that defects in methodology may account for this finding. The method employed was not specific for trypsin but measured total proteolytic activity. It has been shown to be reasonably accurate, however, and changes in trypsinogen concentration in the range of 3–4 per cent can be detected (Beck *et al.* 1962).

It is concluded that, although conversion of very small amounts of

TABLE VI

TRYPSIN AND TRYPSINOGEN IN TRYPSIN DIGESTED BLOOD INDUCED PANCREATITIS OF THREE HOURS' DURATION

(*Five dogs*) (*units/gm. dry tissue*)

	Head	*Body*	*Tail*	*p*
Trypsin	2304	2525	3840	—
Trypsinogen ..	693504	651877	629624	—

— No significant difference in head *vs.* tail ($p = > 0.05$)

trypsinogen cannot be excluded, it appears that progression of the disease is independent of the presence of detectable amounts of free protease. Similar conclusions were found in extensive studies on bile induced pancreatitis (Beck *et al.* 1962).

Conclusions

It is apparent that further biochemical investigations are necessary before Anderson's concept can be accepted that, early in the course of pancreatitis, free trypsin reacts with haemoglobin to form a vasotoxic substance. However, it has been demonstrated clearly elsewhere that bile or trypsin induced pancreatitis is associated with activation of proelastase and activation of this enzyme could explain the vascular damage which occurs.

Trypsin digested blood appears to have an inherent elastolytic activity.

D. THEORY OF THE PATHOGENESIS OF ACUTE HAEMORRHAGIC PANCREATITIS

Acute haemorrhagic pancreatitis develops as the end-result of a continuous and not an abrupt process and there is evidence that, once initiated, the process may be self-perpetuating (Anderson, 1961).

An abnormality occurring in the acinus is usually the initiating factor in an attack of pancreatic oedema. This may result from the combination of ductal obstruction and exocrine hypersecretion (Dreiling, 1961), which leads to an increase in intraductal pressure and leakage of enzymes into the interstitium of the gland (Montaldo, 1954). There is also evidence that zymogens and lysozymes are released (Keith *et al.* 1958, Weissmann 1965). The increase in capillary permeability, which follows, results in loss of fluid and cells into the interstitium and, although these changes can be induced by free trypsin (Anderson and Bergan, 1962), there is no conclusive evidence that this enzyme is released (Beck *et al.* 1962). The inflammatory response results in vasodilatation and an increase in blood flow to the gland. However, because pancreatic oedema develops and the weight of the gland is increased, the perfusion rate is decreased but not to a critical level.

As a result of further progress of the disease, severe oedematous pancreatitis develops. It is possible that further enzymatic activity such as the release of elastase (Geokas, 1966) or the formation of a vasotoxic substance (Anderson, 1961) occurs and leads to further vascular damage and loss of intravascular fluid. A vasculitis occurs which affects the walls of the veins predominantly. It has been demonstrated that the walls of the smaller veins contain an abundance of lysozymes (Movat and Fernando, 1964) and, since the activity of these enzymes may play an important rôle in the pathogenesis of this disease, their presence may explain the occurrence of the most severe vascular changes in this position.

The loss of fluid into the pancreas and the peripancreatic tissues, in addition to the likelihood that blood is trapped in the sinusoids of the liver (Anderson *et al.* 1967), results in marked hypovolaemia which may

approach 35–40 per cent of the plasma volume within a few hours of the onset of the disease (Keith and Watman, 1954). There is frequently a fall in the systemic blood pressure and cardiac output, and further loss of fluid from the intravascular compartment results in stasis of erythrocytes and the formation of haemagglutination thrombi in the capillaries. This, combined with other factors, results in a reduction of the total blood flow to the gland and to a further reduction in pancreatic perfusion rate.

The progression to the final stage of haemorrhagic necrosis will occur if these processes are not reversed and many complicated changes now take place. Hypovolaemia results in reflex splanchnic vasoconstriction (Wiggers, 1950) and this leads to a further decrease in the arterial blood supply to the gland. Stasis in the microcirculation is thus aggravated and further haemagglutination thrombi form. This eventually results in tissue anoxia and a metabolic acidosis, which in turn results in further vasoconstriction and increase in tissue anoxia (Hardaway *et al.* 1966). A vicious circle is thus created which ultimately ends in necrosis and cellular death if it is not broken before it becomes irreversible. Thrombosis in the capillaries, veins and arteries soon follows, and further digestion of capillaries and venules results in disruption and gross interstitial haemorrhage. The total pancreatic blood flow and tissue perfusion are grossly reduced and this results in defective tissue perfusion, parenchymal necrosis and haemorrhage from disrupted pancreatic blood vessels.

E. EFFECT OF DELAYED TREATMENT ON THE DEVELOPMENT OF HAEMORRHAGIC PANCREATITIS

Although it has been demonstrated conclusively that immediate treatment with low molecular weight dextran, Thrombolysin or sympathectomy can reverse the processes described above, acute pancreatitis in man is not seen until after the onset of the disease. Therefore, treatment inevitably is delayed to some extent and it was considered important to determine whether delay in commencing treatment by these agents still had a beneficial effect.

Material and Methods

A potentially lethal acute haemorrhagic pancreatitis was induced in a further 20 dogs by injection of trypsin digested blood into the main pancreatic duct. During and immediately after operation, each dog was given intravenously 60 ml./kg. dextrose/saline solution. This was repeated every 24 hours until death or sacrifice at 72 hours. The following groups of animals were studied.

Heparin group (10 *dogs*): These animals received 10 ml./kg. normal saline every eight hours. Heparin therapy was started eight hours after operation with an intravenous dose of $\frac{1}{2}$ mg./kg. and a subcutaneous

dose of ½ mg./kg. Thereafter, heparin was given in a dose of 1 mg./kg. subcutaneously every 12 hours until sacrifice at 72 hours.

Sympathectomy group (five dogs): These animals received 10 ml./kg. normal saline every eight hours. Eight hours after pancreatitis had been induced, each animal was re-anaesthetized and postganglionic sympathetic denervation of the pancreas performed.

Low molecular weight dextran group (five dogs): These animals received 10 ml./kg. normal saline immediately after pancreatitis had been induced. Twelve hours later, treatment with 1 gm./kg. low molecular weight dextran every eight hours was started.

Results (Table VII)

TABLE VII

ACUTE PANCREATITIS

Effect of Delayed Treatment

Treatment	*No.*	*Mortality*	*Haemorrhagic pancreatitis*			*Oedematous pancreatitis*		
			+++	++	+	+++	++	+
Heparin	10	—	—	1	—	4	4	1
Sympathectomy ..	5	1	—	1	1	2	1	—
L.M.W. dextran ..	5	—	—	—	2	1	1	1

Heparin group: The general condition of each animal was poor when therapy was begun, but within a few hours considerable improvement occurred and all animals were in good condition at the time of sacrifice. There were no deaths. At autopsy, haemorrhagic pancreatitis was present in only one dog and the degree of oedematous pancreatitis in the others was either moderate or severe.

Sympathectomy group: There was one death in this group and haemorrhagic pancreatitis was present in 40 per cent of the animals at autopsy.

Low molecular weight dextran group: None of the animals in this group died. The general condition of each animal was poor at the time treatment was started, but within a few hours considerable improvement had occurred. These dogs had either mild haemorrhagic or a varying degree of oedematous pancreatitis at autopsy.

Comment

The development of haemorrhagic pancreatitis was prevented in the majority of animals by delayed therapy with heparin, sympathectomy or low molecular weight dextran. Heparin prevented thrombosis from occurring, sympathectomy resulted in vasodilatation and an increase in blood flow, whilst low molecular weight dextran prevented stasis and haemagglutination thrombi forming in the pancreatic microcirculation.

These results support the view that the progression of oedematous to haemorrhagic pancreatitis is a continuous process, which takes 12 to 24 hours to complete and which, in the first few hours at least, is reversible. There was a striking reduction in mortality if the process was inhibited within eight or 12 hours of its onset. The morbidity, judged by the clinical course and the macroscopic and microscopic changes in the

pancreas, suggested that there was not complete reversal of all the processes resulting in haemorrhagic pancreatitis, but the majority of the animals were well at the time of sacrifice and it is very probable that most of them would have survived. These studies suggest that effective treatment in humans with acute pancreatitis might still be feasible despite the fact that it cannot be initiated at the onset of the disease process.

F. CLINICAL STUDIES AND FUTURE PROGRESS IN TREATMENT

There has been little attention directed towards correcting the vascular abnormalities which have been shown to occur in pancreatitis. Sympathetic block has been advocated and good relief of pain has been reported (Popper *et al.* 1948). The largest series treated in this manner have been reported by Gage and Gillespie (1951), who claimed good results in 30 patients. Unfortunately all these reports lack essential information and further work is required in this field.

The author has treated 10 severe cases of acute pancreatitis in man with low molecular weight dextran, in addition to other supportive but non-specific therapy. There were no deaths in this small series, and the therapeutic response was very gratifying. This has prompted the author to recommend treating further cases of acute pancreatitis with this substance. The results of an adequate clinical trial must be awaited, however, before a final conclusion can be made.

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