Biochemical Pharmacology, 1967, Vol. 16, pp. 59–72. Pergamon Press Ltd., Printed in Great Britain.

TRASYLOL*

I. TRAUTSCHOLD, E. WERLE and GERTRUD ZICKGRAF-RÜDEL

Klinisch-Chemischen Institut an der Chirurgischen Klinik der Universität München, Munich, Germany

(*Received* 28 *February* 1966; *accepted* 23 *August* 1966)

Abstract—Procedures for the isolation of a kallikrein inhibitor from bovine pancreas and lung (Trasylol) are described. The inhibitor is thought to be present under physiological conditions as a dimer as its molecular weight is twice that calculated from the amino acid composition. pH-related changes in tertiary structure can be shown by changes of the absorption spectrum of the inhibitor. Enzymes inhibited by Trasylol are trypsin, chymotrypsin, kallikrein, plasmin, and to a lesser degree Pronase P and papain. Kinetic data of the inhibition and resorption by the organism as well as distribution in the body are described.

Therapeutic actions of Trasylol include: (1) inhibition of locally activated kininogenases which liberate kinins; (2) inhibition of the activation of the proteolytic "spontaneous activity" in pancreas homogenates; (3) decrease in secretory rate, and secretion of pancreatic enzymes and (4) inhibition of kininogenases in the vascular system, thereby preventing the liberation of kinins which cause circulatory shock.

THE USE of antimetabolites and inhibitors of enzymes for therapeutic purposes has increased in recent years. According to its specificity, the trypsin-kallikrein inhibitor, Trasylol, can bring back pathologically increased proteolysis to normal levels.

Forty years ago Frey[22] demonstrated the presence of a high-molecular depressor substance in human urine which was later called kallikrein (because a similar substance was present in the pancreas). A substance capable of inhibiting kallikrein was first found in human blood by Frey and Kraut[23, 44a] and was named kallikrein inactivator. Three years later a second kallikrein inhibitor was demonstrated in the parotid gland, spleen, liver, and lymph nodes of cattle by Frey *et al.*[7, 45] Subsequently, Kunitz and Northrop[51] isolated a trypsin inhibitor from bovine pancreas, and Astrup[5] found a substance in bovine lung that inhibits fibrinolysis.

Werle *et al.*[98] showed that the kallikrein inhibitor from bovine lymph nodes and parotid gland also inhibits trypsin and chymotrypsin. That the pancreatic trypsin inhibitor of Kunitz and Northrop can inhibit kallikrein from serum, pancreas, submandibulary gland, and urine of most mammals was shown by Trautschold and Werle.[85, 86] In addition Werle[100, 104] demonstrated that the inhibitory substance from bovine lung has the same inhibitory specificity as the kallikrein-trypsin inhibitor from bovine parotid gland, lymph nodes, liver, and spleen. Finally, together with Marx, Werle found that these inhibitors can also inhibit plasmin.[58, 59, 99]

This kallikrein-trypsin inhibitor exists only in certain ruminants, and is especially abundant in various organs of the ox. It is different from the selective trypsin inhibitor

* Farbenfabriken Bayer A.G., Leverkusen, Germany.

present in the pancreas of man, dog, pig, ox, and probably all mammals; from the trypsin and chymotrypsin inhibitor present in the submandibulary gland of the dog; and from the trypsin inhibitor of the accessory sex glands of mammals.[25, 32, 89] For example, the kallikrein-trypsin inhibitor is not secreted into the parotid secretion or the pancreatic juice, whereas the specific pancreatic trypsin inhibitor is found in low concentration in the pancreatic juice. Furthermore, the inhibitory spectrum of the serum inhibitors differs from that of the proteinase inhibitors derived from bovine organs (Table 1).[90]

The tissues that contain the highest concentration of kallikrein-trypsin inhibitor are bovine parotid gland and bovine lung, with 300 to 400 KIU* per gram and 1000 KIU per gram respectively (which corresponds to 170 to 230 ImU and 575 ImU respectively).[24, 104] These tissues, therefore, constitute especially rich sources for isolation purposes.

TABLE 1. INHIBITORY SPECTRUM OF NATURALLY OCCURING INHIBITORS

Source	Trypsin	Chymotrypsin	Mammalian kallikreins			Plasmin
			Serum	Pancreas, submandibulary gland	Urine	
Serum (human)						
α_1	+	+	−	−	−	−
Inter-α	+	−	−	−	−	−
α_2			−	−	−	+
Postalbumin	−	+	−	−	−	−
α	−	−	+	+	+	−
β	−	−	+	+	+	−
Bovine organs						
Pancreas (Kunitz)	+	+	+	+	+	+
Parotid	+	+	+	+*	+†	+
Lung	+	+	+	+	+	+
Pancreas (mammalian)	+	−	−	−	−	−
Submandibulary gland (dog)	+	+	−	−	−	−

* Except dog and rat pancreas.
† Except dog.

PROCEDURES FOR ISOLATION

Procedures for isolation of the kallikrein-trypsin inhibitor include alcohol fractionation and paper electrophoresis,[46–48] and cellulose ion-exchange chromatography.[90] The inhibitor can be crystallized by salting out.[49, 79] In the crystalline state, and also after chromatographic purification, the activity of 1 KIU corresponds to 0·14 to 0·15 μg of organic substance. The kallikrein-trypsin inhibitor from bovine lung has been produced industrially in almost pure form on a large scale and is available commercially as Trasylol®.

* Since kallikrein was the first enzyme found to be inhibited by Trasylol, the KIU (kallikrein inhibitor unit) is defined as that amount of inhibitor which under certain specific conditions produces 50 per cent inhibition of 2 KU (kallikrein units, biologically determined). As most inhibitors for proteolytic enzymes also inhibit trypsin, we propose to define the inhibitory unit generally on the basis of trypsin inhibition. This so-called international inhibitor unit (IU) on the basis of trypsin inhibition is that amount of inhibitor (also of Trasylol) which, under standard conditions, inhibits the activity of 1 international unit of trypsin activity; that is, the splitting of 1 μmole substrate, e.g. of BAPA (benzoylarginine *p*-nitroanilide), per min. This definition of IU may be used for all known synthetic substrates for proteolytic-esterolytic enzymes.

STRUCTURE AND PROPERTIES

There is considerable evidence that no essential difference exists between the kallikrein-trypsin inhibitors from various bovine organs.[3, 50, 79] The kallikrein-trypsin inhibitor from bovine lung is a basic polypeptide composed of 58 amino acids. The amino acid sequence of the inhibitors from parotid gland and lung of beef has been shown[1, 2] to be the same as that found for the pancreatic trypsin inhibitor discovered by Kunitz (Fig. 1).[41] The molecular form in which the naturally occurring inhibitor

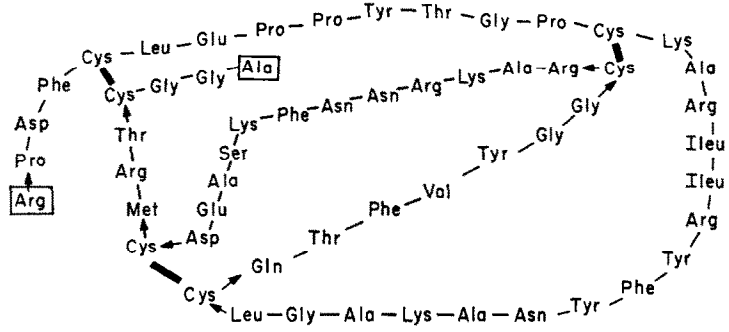

FIG. 1. Amino acid sequence of the Kunitz inhibitor from pancreas (after Kassell *et al.*[41]).

exists has not yet been fully elucidated. The molecular weight of 11,600 determined by Kraut and associates[48] from the sedimentation constant was also found by Anderer[1] in preparations studied under physiological conditions. If the molecular weight 6511 calculated from the amino acid sequence is taken as a basis, the inhibitor must be present largely as a dimeric aggregate, the mean molecular weight of 10,300 to 11,800 being attributed to the presence of a small amount of the monomeric component. It may be that the association of two inhibitor molecules is mediated by a lower molecular connecting link.[1] When the molecular weight of the kallikrein-trypsin inhibitor is determined by gel filtration, complete dissociation of the inhibitor aggregates occurs, giving a molecular weight of 6700 $\pm$ 200.[26, 90] Because of its low molecular weight, the inhibitor is dialyzable and cannot be precipitated by deproteinating agents.

The isoelectric point is pH 10·5. In a neutral and, in in particular, in an acid milieu, the inhibitor is remarkably stable, as it is also at high temperatures. Even in an alkaline milieu it is relatively stable.[90]

At pH 5·9 the ultraviolet absorption spectrum has a maximum at 280 mμ and a minimum at 250 mμ. Detailed information on pH-related changes in tertiary structure can be obtained from the differential spectra. With increasing alkalinity, the tyrosyl groups come to the surface and dissociate. In strongly alkaline conditions there occurs an irreversible change in molecular structure, which is expressed as an abnormal absorption spectrum. Partial and reversible denaturation also takes place on addition of urea.[90]

ENZYMES INHIBITED

The inhibitor inhibits enzymes of different substrate specificity, which have in common esterolytic and proteolytic, and in some cases kinin-releasing, activity. Such enzymes are trypsin, chymotrypsin, various kallikreins, plasmin, certain bacterial proteinases from *Streptomyces griseus*, and some cell proteinases not yet defined.

Trypsin

The stoichiometric reaction of trypsin with Trasylol is pH-dependent above pH 11 and below pH 6 and is reversible. Proteolytic, esterolytic, and kinin-releasing activities are inhibited to the same degree. With trypsin, as with the other enzymes described below, inhibition increases linearly up to limit values of 70 to 90 per cent. Complete inhibition is accomplished only by an inhibitor excess. In the physiological pH range, theoretically a 100 per cent inhibition of 0·56 to 0·7 μg trypsin is achieved by 1 KIU, whereas actually 1·2 KIU is necessary for complete inhibition. When BAPA is used as substrate, 1 ImU is bound to 0·27 μg of inhibitor (calculated from 50 per cent inhibition; see footnote*).

The dissociation constant of the trypsin inhibitor complex is extremely low. Yet the inhibition of trypsin is a time reaction and requires 4 min to reach equilibrium when a 10^{-7} M enzyme solution is used at 25°. The inhibition is not competitive, provided the trypsin substrate (BAPA: $K_m = 4{\cdot}3 \times 10^{-4}$ mole/l.) is added after formation of the trypsin inhibitor complex; if the substrate and the inhibitor are added at the same time, competitive inhibition results. The extrapolated inhibitor constant at pH 7·8 is 2×10^{-11} mole/l. With decreasing pH value the dissociation of the complex increases; that is, the inhibitor constant becomes greater (for example, at pH 4·0 $K_i = 2{\cdot}6 \times 10^{-9}$ mole/l.).[90]

Chymotrypsin

Because of the similarity between the properties of trypsin and chymotrypsin, what has been said about inhibition of trypsin applies also to a large extent to inhibition of chymotrypsin. When SUPHEPA* ($K_m = 2{\cdot}5 \times 10^{-3}$ mole/l.) is used as substrate, 1 KIU inhibits the activity of 0·44 μg chymotrypsin. The ratio of trypsin inhibition to chymotrypsin inhibition is 1 : 0·68, based on equal weights. The chymotrypsin inhibitor complex also dissociates in an acid milieu, although the dissociation constant at corresponding pH values is significantly greater than that for the trypsin inhibitor complex. In a mixture of trypsin and chymotrypsin it is trypsin that is preferentially inhibited by Trasylol.[90]

Kallikrein

First a brief word about the kallikreins. It was their hypotensive action that first drew attention to the presence of a kallikrein in the urine.[22] Later their presence was demonstrated by Frey *et al.*[24] in the form of the inactive precursor (prekallikrein) in the pancreas and blood plasma and, in an active form, in the submandibulary glands of all the mammals studied. The kallikreins are enzymes which exert a strictly specific proteolytic activity against an α_2-globulin fraction of the serum (kininogen). They also possess an esterolytic action on certain synthetic amino acid esters of the type of BAEE.† It was at first thought that the kallikreins themselves lower the blood pressure, but Werle *et al.*[96, 97] showed that the depressor response depends on the enzymatic release of kinins from the serum precursor kininogen. In the same way trypsin acts as a depressor agent by kinin release. The pharmacological actions of the kinins include stimulation of smooth muscle, peripheral vasodilatation, enhancement of capillary permeability, and production of pain (literature review, Trautschold and Rüdel[88]).

* SUPHEPA = Succinyl-phenyl-*p*-nitroanilid.

† BAEE = Benzoyl-arginine-ethyl ester.

The kallikreins differ from each other in certain physico-chemical properties and with regard to their ability to liberate kinins. It has been shown by Werle *et al.*, Webster, and Habermann that the kallikreins from mammalian pancreas, submandibulary gland, and urine release the decapeptide kallidin from serum or from purified kininogen preparations, while serum kallikrein, like trypsin and certain snake venoms, releases the nonapeptide bradykinin. (The relevant literature has been reviewed by Trautschold and Rüdel.[88]) The inhibitor considered here can inactivate the kinin-releasing (proteolytic) and esterolytic activity of all known kallikreins, except pancreatic kallikrein of dogs and rats, and urinary kallikrein of dogs.[105] The trypsin inhibitor from soybean inhibits only the serum kallikreins (literature review, Vogel *et al.*[94]). Inactivation of the kallikreins may be determined biologically in terms of reduction of their action on the blood pressure[24] or in terms of inhibition of their esterolytic action on synthetic amino acid esters such as, for example, BAEE[86, 87]

In measuring the inhibitory activity of the kallikrein-trypsin inhibitor by its lowering of the splitting rate of BAEE by hog pancreatic kallikrein, for instance, 1 KIU corresponds to 0·06 to 0·07 IU. Even in the presence of BAEE the inactivation is not competitive. When this inhibition of kallikrein is determined, it must be remembered that the degree of inactivation should not significantly exceed 50 per cent, otherwise the proportionality of the inactivation is no longer assured. Even at a KU:KIU ratio of 1:6, not more than 95 per cent of the kallikrein is inactivated.

This incomplete inhibition is explained by the relatively high dissociation constant of the kallikrein inhibitor complex (at pH 7·8 $K_i = 1{\cdot}2 \times 10^{-8}$ mole/l.). At pH 4·0 the kallikrein inhibitor complex is already completely dissociated. Since the inhibitor is more firmly bound to trypsin than to kallikrein, if trypsin is added to the kallikrein inhibitor complex it displaces kallikrein from its binding to the inhibitor. Once formed, the trypsin inhibitor complex is not split on addition of kallikrein even in great excess.[90]

A solution of the kallikrein inhibitor complex obtained from the pure components may contain up to 5 per cent of its kallikrein in unbound form. On intravenous administration, such a solution represents a depot form of kallikrein from which active kallikrein is developed by dissociation. Hitherto, splitting of the complex *in vivo* has been demonstrated by pharmacological methods only, namely, by observing a prolonged depressor effect[27] (G. Kroneberg, personal communication). A proof for the dissociation of the complex *in vivo* is the demonstration of the free inhibitor in the liver and the kidney. In spite of its higher stability, the trypsin inhibitor complex is split more quickly than the kallikrein inhibitor complex. This is due to the remarkably high concentration of the trypsin inhibitors of the blood plasma, which is higher to the tenth power than that of the kallikrein inhibitor. The trypsin molecules released by dissociation are immediately bound by these trypsin inhibitors

Plasmin

In 1959 the effect of Trasylol in hyperfibrinolysis in streptokinase-activated blood was first demonstrated.[59] Only the inhibition of plasmin in a purified test system has been demonstrated; the effect on streptokinase and the related activator system still requires further experimental study (Trasylol Symposium,[84]). When measured by the splitting of synthetic substrates, 1 KIU inhibits the activity of 6 μg plasmin.*

* Plasmin Novo: 1 μg enzyme protein corresponds to 1 ImU (BAEE).

Pronase

Pronase-P*) is a mixture of proteinases from *Streptomyces griseus* which also is capable of splitting ester and amide bonds of synthetic substrates. When measured in terms of splitting of BAEE, 1 KIU inhibits 3·75 μg of the enzyme preparation.

Papain

Trasylol produces very little inhibition of papain (1 KIU inhibits 0·002 μg papain).

Pepsin

Pepsin is neither inhibited by Trasylol nor is Trasylol degraded by pepsin.[90]

DISTRIBUTION AND EXCRETION OF THE INHIBITOR

Accurate control of Trasylol blood levels during therapy was first achieved by Werle and Trautschold,[102] who developed a method for quantitative determination of Trasylol in tissues and blood. The high blood levels needed for effective therapy can be attained only by intravenous administration. Blood concentrations after intravenous, intraperitoneal, intramuscular, and subcutaneous administration of equal amounts of inhibitor decrease in that order. No absorption follows oral administration (even as capsules soluble in the small intestine) or rectal administration as suppositories.

With a single intravenous injection of 1000 to 2000 KIU/kg, the half-life was about 10 min. Because of this rapid elimination from the blood stream, the best method of administration for therapeutic purposes is by continuous intravenous drip. Trautschold and Werle[89] studied the distribution in the organs of the rat after intravenous injection of 12,000 KIU/kg. They found that the inhibitor is at first demonstrable in small amounts in all the organs, but that it subsequently becomes selectively deposited in the liver, where it reaches a maximum 30 min after injection. After a latent interval of about 10 min, the inhibitor content in the renal tissue begins to rise, and after an hour is higher than that of the liver in a proportion of 50 to 36 per cent. At the same point of time not more than about 10 per cent is present in the blood stream. Four to five hours after the injection, almost the whole amount of inhibitor administered is found in the renal tissue in active form.

The results suggest that the inhibitor undergoes structural changes in the liver which condition it for fixation in the kidneys, without its inhibitory activity being lost in this process. Using tritium-labeled Trasylol, Trautschold and his associates[89] showed that over several days only a small part of the inhibitor is excreted in biologically active form, the bulk being demonstrable in the urine as tritium activity only. The degradation of the inhibitor occurs in renal tissue. The inhibitor is excreted only via the kidneys.[6, 40, 89]

TOXICITY

One of the characteristics of Trasylol is its excellent tolerance even at high dosage. The LD_{50} in mice is 2·5 million KIU/kg.[11] In dogs, intravenous administration of up to 1 million KIU/kg is tolerated without complications. High doses are less well tolerated in rats, in which—especially in animals which have undergone surgery—they

* California Corp. for Biochemical Research, Los Angeles.

may lead to fatal shock-like reactions.[12] Very rarely, repeated use of extremely high doses has led to symptoms of intolerance in patients with a history of allergy.[11] The extensive use of Trasylol in therapy cannot be discussed here.

ACTION OF TRASYLOL IN PANCREATITIS

The events taking place in acute pancreatitis are set out schematically in Fig. 2. The primary reaction to unphysiological stimulus of the pancreas is the formation of

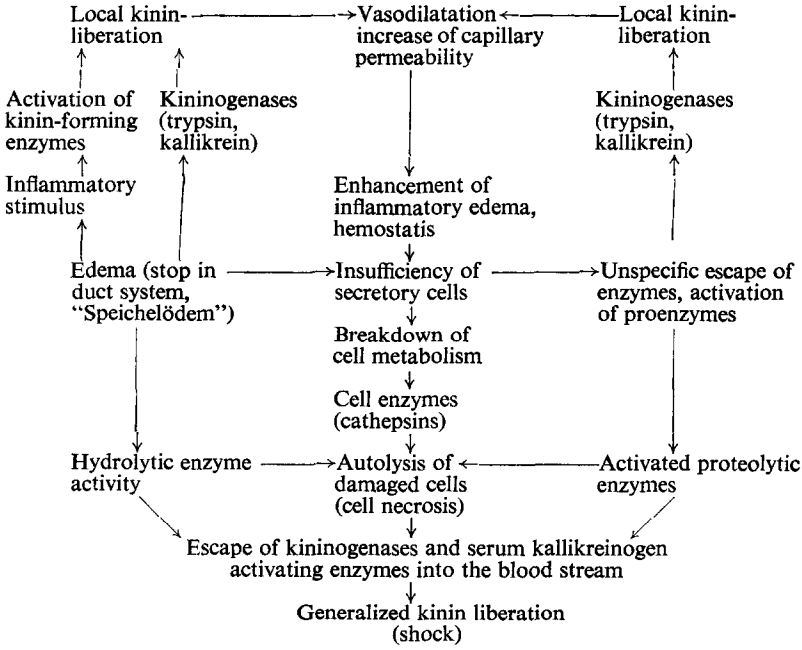

FIG. 2. Scheme of the pathophysiological reactions in the course of acute pancreatitis.

edema.[106, 107] According to Becker,[10] the edema is the result of penetration of pancreatic juice through intact duct epithelium, a process which requires prior obstruction of the duct and stimulation of secretion. According to Doerr,[16–18] the damming of pancreatic secretion leads to secretion "in the wrong direction," that is, into the interstitial tissue where it has an inflammatory effect. The acinar cells become deficient in energy, a state that is at first reversible. Subsequent. capillary damage and the resultant escape of fluid from the vascular system contribute to extension of the edema.

The extent to which the edema is reversible depends on the possibilities of absorption via the blood stream and the lymphatic system.[19] The edema fluid contains the inactive precursors of the kinin-liberating enzymes serum kallikrein, pancreatic kallikrein, and trypsin, as well as kininogen. The prekallikrein of the serum can be activated in an unspecific manner by any inflammatory process. The activation process of pancreatic prekallikrein and trypsinogen is not yet fully elucidated. Pancreatic kallikrein, however, is demonstrable in active form in tissue homogenates. [89] Under the influence of serum and pancreatic kallikrein (and theoretically also under the influence of trypsin), kinins are released, at first locally. This in turn promotes the

pathological process, because the kinins increase vascular permeability (therby intensifying the exudative stage) and dilate the capillaries (thereby increasing stasis in the local vascular bed). The arrest of these processes by inhibition of local release of kinins is the *first point* at which Trasylol exerts a therapeutic action (Fig. 3).

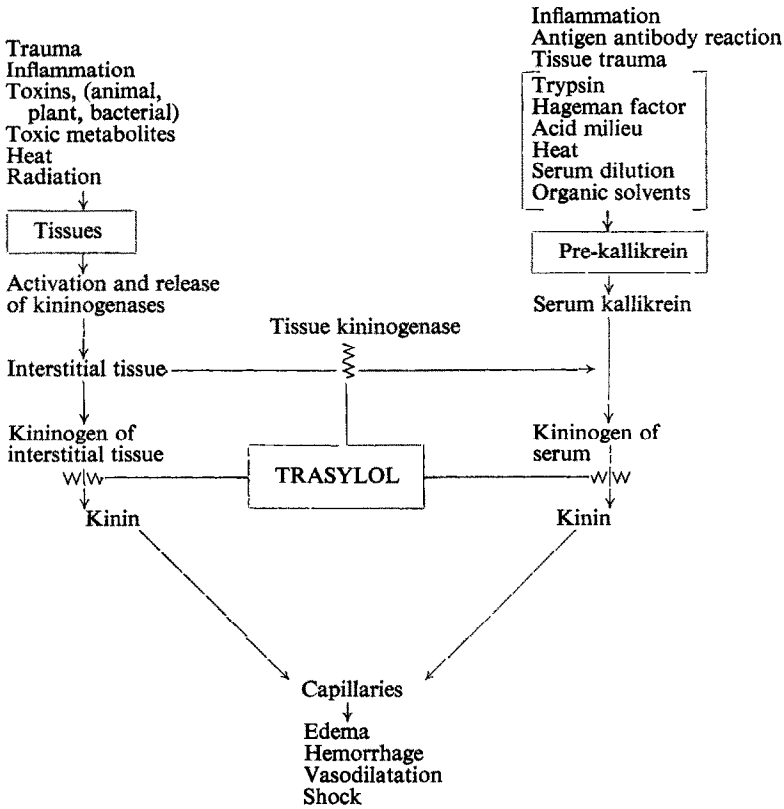

FIG. 3. Points at which Trasylol inhibits the formation of kinin.

In addition to damage of the acinar cells resulting from the damming of secretions, and the relative hypoxia which accompanies extremely high metabolic activity, there also occurs a disorder of supply that is related to the edema, and which results from impeded exchange of metabolites and from injury to the capillary system. If the insufficiency of the cells increases, the damage becomes irreversible.

Side by side with these processes there occurs activation of the proteolytic enzyme components which induces autolysis of the damaged cells. In what the initial activation consists is not yet known. It may start from the "secretion edema" or from the damaged cells. The unique role in necrosis of the pancreas that was formerly attributed to trypsin could never be proved experimentally. Haverback *et al.*[35] found that neither spontaneous nor autocatalytic activation of trypsinogen occurs in pancreatic secretion. When pancreatic tissue was studied during autolysis a fall in trypsinogen level was observed in some cases.[74, 76, 89] Only after a long interval from the onset of autolysis in pancreas homogenates (species-dependent; e.g. at least 7–12 hr for rat pancreas), a tryptic activity is demonstrable by means of synthetic substrate (BAPA) which can be

inhibited by Trasylol and soybean-trypsin inhibitor (H. Haendle, I. Trautschold and E. Werle, unpublished).

The difficulty in demonstrating active trypsin is caused partially by the presence of the specific trypsin-inhibitor of the pancreas which binds the active trypsin immediately. In the writers' opinion trypsin may appear in the role of a primary activator in microareas of the cells, since it is still thought to be the only activator of the other zymogens of the pancreas. During autolysis, partial activation of kallikrein[89] and carboxypeptidase is demonstrable.[74, 76] In very early stages of autolysis, a caseinolytic activity (so-called spontaneous activity), which cannot be inhibited by Trasylol and other trypsin inhibitors is already demonstrable, but Trasylol can inhibit its rise in the course of autolysis.[67, 68, 75, 90] In addition a catheptic enzyme is active in the homogenates.[90]

The *second point* of action of therapeutic Trasylol is partial inhibition of the reactions which lead to activation of the proteolytic system.

The *third* therapeutic action of Trasylol, according to Schultis and Rick,[78] and Schönbach *et al.*,[77] is that it reduces the secretion rate by the excretory cells, thereby reducing not only the output of proteinases which are capable of undergoing activation and of all other pancreatic enzymes, but also the metabolic activity of the parenchymal cells. Schönbach and associates could show that the trypsin content in pancreatic juice is lowered before reduction of the other pancreatic enzymes and secretion volume.

As micro- and macronecrosis develops, pancreatic enzymes enter the blood stream in increasing amounts and lead to generalized formation of kinins which may be proved indirectly by a fall in kininogen blood level.[33, 39, 42, 72, 73, 83, 91] Dependent on the velocity by which kinins are released and on the amount of active kinins in the circulating blood, the blood pressure is lowered. Continuous and slow release of kinins has little effect on the blood pressure, as active kinins are rapidly destroyed by kininases in the serum. [24, 101] Uncontrolled and generalized formation of kinins is one of the factors causing vascular collapse, which frequently is observed in acute pancreatitis.

This points to the *fourth*, and perhaps the most significant, mode of action of Trasylol, namely, arrest of the peripheral circulatory reactions and consequent prevention of the harmful effects of reduced blood flow and other circulatory derangements of the pancreas and of renal function.

The therapeutic effectiveness of Trasylol in experimental pancreatitis has been studied chiefly in the dog and the rat. The essential criteria of the therapeutic effects are, in comparison with untreated controls, an increased survival rate (Arzt,[4] Falcidieno,[20] Forell,[21] Grözinger,[28,29] Haig,[33] Hoferichter,[36] Kelly,[43] Maleki,[55] Nabseth,[65] Nemir,[69] Nugent,[70] Ribacoff,[71] Smith,[82] Tsukiyama[92]); less elevation of blood amylase and lipase levels (Arzt,[4] Grözinger,[28] Hatano,[34] Hoferichter,[36,37] Kelly,[43] McHardy,[54] Mallet-Guy,[56, 57] Tsukiyama[93]); reduced edema and fewer histologically demonstrable destructive changes in the pancreas (Falcidieno,[20] Forell,[21] Grözinger, [28–30] McCutcheon,[52–53] McHardy,[54] Mallet-Guy,[56, 57] Ribacoff,[71] Tsukiyama[93]).

Only in a minority of cases have all these criteria of the positive effects of Trasylol been present at the same time. In a series of studies, no significant therapeutic action could be demonstrated.[9, 12, 13, 80, 81]

Trasylol should be given in early states of pancreatitis in order to arrest the local effects of the release of kininogenases. This recommendation is experimentally

supported by the beneficial results in experimental pancreatitis in animals premedicated with Trasylol or given Trasylol immediately after onset of pancreatitis.[4, 28, 29, 43, 54, 69, 92, 93]

Differences in experimental arrangement (concerning, for example, the degree of severity of the pancreatitis, the dosage of Trasylol, the time of its administration, additional medication, general and nutritional state of the animals used) make it difficult to compare the results obtained by the various research groups. Experimental pancreatitis in the dog and the rat does not, at least with regard to therapy with Trasylol, reflect what happens in man, for in these animals Trasylol does not inhibit pancreatic kallikrein *in vivo* and *in vitro* as it does in the human subject. Consequently in these animals, the local and (possibly) general releast of kinins by activated pancreatic kallikrein continues unchecked by Trasylol, although this, as we have seen, is one of the points at which the drug can take effect. Since experimental studies indicate that in pancreatitis Trasylol does not act (or acts only to a minor extent) on trypsin, its only mechanism of action in the dog and the rat, apart from influencing "spontaneous activity" and secretion rate, must be inhibition of the serum kallikrein, which can be activated by nonspecific mechanisms, and consequent release of kinins.

However, the fact that Trasylol exerts a positive therapeutic effect in the dog and the rat suggests that even the initial intrapancreatic processes that can be inhibited by Trasylol depend on the activity of serum kallikrein, or that the chief action of this drug is inhibition of the peripheral release of kinins by serum kallikrein. In human pancreatitis the processes induced by pancreatic kallikrein are also inhibited.

Despite the large number of clinical reports available, it is difficult to make a statistical evaluation of the therapeutic effects of Trasylol in human pancreatitis. Comparisons in animal experimentation are difficult enough, but in man there is the additional factor—which is mainly responsible for variations of the results in the different studies—that there is no clinical method for exact determination of the severity of the disease condition.[38, 61] Aside from the conflicting data on the effect of Trasylol on mortality, there is no doubt that its administration is followed by almost immediate relief of pain and by improvement of the circulatory state during the acute stage of shock.[8, 35a] Although pain production is a propertyof the kinins, relief of pain by Trasylol is probably due to its reducing edema and thereby easing the painful capsular tension. The part played by kinins in the circulatory shock has been demonstrated indirectly in acute pancreatitis on the basis of a fall in the kininogen level (E. Werle and E. Asang, unpublished). The fall in blood pressure that follows induction of experimental shock in animals[14, 15, 62, 95, 103] is accompanied by a demonstrable fall in kininogen level. If the animals are premedicated with Trasylol these phenomena are largely suppressed, and if it is given at a later stage the circulatory status is still more rapidly restored to normal. Trasylol also has a beneficial effect on manifestations of shock supervening in the course of other inflammatory processes such as peritonitis.[63, 64, 66]

There is no strict dosage scheme for Trasylol, for dosage must be regulated by the clinical picture. As it is well tolerated up to one million units, and more can be given in the acute stage of pancreatitis or other conditions. In most cases it is sufficient to tart with 50,000–100,000 KIU and to follow this with a continuous drip infusion of up to 50,000 KIU/hr for up to 12 hr. The usual conservative measures should be

taken in addition. In cases of suspected pancreatitis Trasylol should be administered without delay, pending establishment of a diagnosis.

Postoperative pancreatitis and chronic recurrent pancreatitis should be treated with Trasylol during the acute stage. Trasylol is also indicated in pathological conditions, and in surgical interventions in the upper abdomen, in injuries liable to impair the integrity of the pancreas, and in peritonitis.

ACTION OF TRASYLOL IN OTHER PATHOLOGICAL CONDITIONS

Since Trasylol also inhibits plasmin, Marx together with Werle[59] proposed its use as an inhibitor agent in hyperfibrinolysis of various origin. It is supposed to inhibit plasminogen activation too. This therapeutic potentiality extends to all conditions in which the relatively labile equilibrium between fibrinolysis and the thromboplastic system is disturbed and in which the natural inhibitory system does not provide adequate control of the proteolytic processes involved (Trasylol Symposium[84]). Chief indications for the use of Trasylol in this connection are obstetrical, but they also include gynecological and general surgical interventions; positive results have been reported in numerous clinical studies. Special problems, such as hemorrhage due to hyperfibrinolysis following extracorporal circulation, are accessible to therapy by Trasylol. It also may be considered for use as an antidote in thrombolytic therapy with streptokinase.[60]

Recent studies have shown that in very high dosage Trasylol may also inhibit the thrombin system. The mechanism of preventing the action of the thrombus-forming principle is not yet known.[84]

Trasylol is also used to treat tissue damage resulting from mechanical or thermic causes. Here its action is directed both against unphysiological activation of the clotting system and against proteolytic processes leading to liberation of kinins.

It has been observed recently, in animal experimentation, that Trasylol can prevent abdominal adhesions.[31, 44] Here again the mechanism of action has not yet been elucidated.

REFERENCES

1. F. A. Anderer and S. Hörnle, *Z. Naturforsch.* **20b**, 457 (1965).
2. F. A. Anderer, *Z. Naturforsch.* **20b**, 462 (1965).
3. F. A. Anderer, *Z. Naturforsch.* **20b**, 499 (1965).
4. C. P. Arzt, Discussion in P. Nemir, J. Hoferichter and D. L. Drabkin, *Ann. Surg.* **158**, 655 (1963).
5. T. Astrup, 18th Int. Physiol. Congress, Copenhagen, *Abstracts*, p. 81 (1950).
6. J. Auhagen, Dissertation, Med. Fakultät, Düsseldorf (1964).
7. E. Bauer, E. K. Frey, H. Kraut, F. Schultz and E. Werle, *Naturwissenschaften* **17**, 672 (1930).
8. Bayer AG Farbenfabriken, Leverkusen, *Pharma-wiss. Lit.-Abt.* (1965) for references.
9. I. T. Beck, R. D. McKenna, B. Zylberszac, J. Solymar and S. Eisenstein, *Gastroenterology* **48**, 478 (1965).
10. V. Becker, *Sekretionsstudien am Pankreas.* Thieme, Stuttgart (1957).
11. H. Beuchelt, *Medizin und Chemie* **7**, 763 (1963).
12. W. Creutzfeldt, H. D. Söling and H. Ketterer, *Klin. Wschr.* **41**, 1002 (1963).
13. W. Creutzfeldt, H. Schmidt and I. Horbach, *Klin. Wschr.* **43**, 15 (1965).
14. C. R. Diniz, I. F. Carvalho, J. Ryan and M. Rocha e Silva, *Nature, Lond.* **192**, 1194 (1961).
15. C. R. Diniz and I. F. Carvalho. *Ann. N.Y. Acad. Sci.* **104**, 77 (1963).

16. W. DOERR, *Verh. dt. Ges. Path.* **37**, 292 (1953).
17. W. DOERR, *Arch. klin. Chir.* **292**, 552 (1959).
18. W. DOERR, P. B. DIEZEL, K. H. GRÖZINGER, H. G. LASCH, W. NAGEL, J. A. ROSSNER, M. WANKE and F. WILLIG, *Klin. Wschr.* **43**, 125 (1965).
19. A. DUPREZ, S. GODART, R. PLATTEBORSE and J. M. DUPONT, in N. HENNING, K. HEINKEL and H. SCHÖN, *Pathogenese, Diagnostik, Klinik und Therapie der Erkrankungen des exokrinen Pankreas*, p. 207. Schattauer, Stuttgart (1964).
20. P. FALCIDIENO, T. GALLI and E. NICORA, *Pathologica* **54**, 77 (1962).
21. M. M. FORELL and W. DOBOVICNIK, *Klin. Wschr.* **39**, 47 (1961).
22. E. K. FREY, *Arch. klin. Chir.* **142**, 663 (1926).
23. E. K. FREY, *Arch. klin. Chir.* **148**, 643 (1927).
24. E. K. FREY, H. KRAUT and E. WERLE, *Kallikrein (Padutin)*. Enke, Stuttgart (1950).
25. H. FRITZ, I. TRAUTSCHOLD, G. HARTWICH and E. WERLE, Federation of European Biochemical Soc., Second Meeting, Vienna (1965).
26. H. FRITZ, I. TRAUTSCHOLD and E. WERLE, *Hoppe-Seyler's Z. physiol. Chem.* **342**, 253 (1965).
27. K. GOLENHOFEN, H. HENSEL and J. PAPENBERG, in *Leistungen und Ergebnisse der neuzeitlichen Chirurgie*, p. 49. Thième, Stuttgart (1958).
28. K. H. GRÖZINGER and C. P. ARZT, *Arch. klin. Chir.* **305**, 150 (1964).
29. K. H. GRÖZINGER, C. P. ARZT, A. U. HOLLIS and R. L. WESSON, *Surgery* **56**, 400 (1964).
30. K. H. GRÖZINGER and W. WENZ, *Gastroenterologia* **3**, 77 (1965).
31. E. GRUNDMANN, in (Eds. R. GROSS and G. KRONEBERG) *Neue Aspekate der Trasylol-therapie*, p. 178. Schattaeur, Stuttgart (1966).
32. H. HAENDLE, H. FRITZ, I. TRAUTSCHOLD and E. WERLE, *Hoppe-Seyler's Z. physiol. Chem.* **343**, 185 (1965).
33. T. H. B. HAIG and A. G. THOMPSON, *Can. J. Surg.* **7**, 97 (1964).
34. M. HATANO, J. F. GRENIER, M. ADLOFF and A. G. WEISS, *C.r. Séanc. Soc. Biol.* **157**, 1505 (1963).
35. B. J. HAVERBACK, B. DYCE, H. BUNDY and H. E. EDMONDSON, *Am. J. Med.* **29**, 424 (1960).
35a. K. HEINKEL, *Verh. dtsch. Ges. Inn. Med.* **70**, 810 (1964).
36. J. HOFERICHTER, P. NEMIR and D. L. DRABKIN, *Surg. Forum* **13**, 309 (1962).
37. J. HOFERICHTER, in N. HENNING, K. HEINKEL and H. SCHÖN, *Pathogenese, Diagnostik, Klinik und Therapie der Erkrankungen des exokrinen Pankreas*, p. 179. Schattauer, Stugart (1964).
38. J. HOFERICHTER, *Arch. klin. Chir.* **307**, 319 (1964).
39. M. HOLLENBERG, E. E. KOBOLD, R. PRUETT and A. P. THAL, *Surg. Forum* **13**, 302 (1962).
40. H. KALLER, in N. HENNING, K. KEINKEL and H. SCHÖN, *Pathogenese Diagnostik, Klinik und Therapie der Erkrankungen des exokrinen Pankreas*, p. 307. Schattauer, Stuttgart (1964).
41. B. KASSELL, M. RADICEVIC, M. J. ANSFIELD and M. LASKOWSKI, *Biochem. biophys. Res. Commun.* **18**, 255 (1965).
42. W. KATZ, M. SILVERSTEIN, E. E. KOBOLD and A. P. THAL, *Archs Surg.* **89**, 322 (1964).
43. T. R. KELLY, E. P. BRATCHER and W. H. FALOR, *Archs. Surg.* **89**, 317 (1964).
44. E. KERN and C. KUHBIER, *Ergebn. Chir. Orthop.* **46**, 48 (1964).
44a. H. KRAUT, E. K. FREY and E. BAUER, *Hoppe-Seyler's Z. physiol. Chem.* **175**, 97 (1928).
45. H. KRAUT, E. K. FREY and E. WERLE, *Hoppe-Seyler's Z. physiol. Chem.* **192**, 1 (1930).
46. H. KRAUT and R. KÖRBEL-ENKHARDT, *Hoppe-Seyler's Z. physiol. Chem.* **309**, 243 (1957).
47. H. KRAUT and R. KÖRBEL-ENKHARDT, *Hoppe-Seyler's Z. physiol. Chem.* **312**, 161 (1958).
48. H. KRAUT, W. KÖRBEL, W. SCHOLTAN and F. SCHULTZ, *Hoppe-Seyler's Z. physiol. Chem.* **321**, 90 (1960).
49. H. KRAUT, N. BHARGAVA, F. SCHULTZ and H. ZIMMERMANN, *Hoppe-Seyler's Z. physiol. Chem.* **334**, 230 (1963).
50. H. KRAUT and N. BHARGAVA, *Hoppe-Seyler's Z. physiol. Chem.* **338**, 231 (1964).
51. M. KUNITZ and J. H. NORTHROP, *J. gen. Physiol.* **19**, 991 (1936).
52. A. D. MCCUTCHEON and D. RACE, *Ann. Surg.* **158**, 233 (1963).
53. A. D. MCCUTCHEON and D. RACE, *Ann. Surg.* **160**, 1041 (1964).
54. G. MCHARDY, C. C. CRAIGHEAD, L. BALART, H. CRADIC and C. LA GRANGE, *J. Am. med. Ass.* **183**, 527 (1963).
55. M. F. MALEKI, W. F. COULSON and E. ENGLERT, unpublished, Farbenfabriken Bayer, *Pharmawiss. Lit. Abt.* (1965).

56. P. MALLET-GUY, C. BOSSER, J. MICHOULIER, D. TOPIS, J. FEROLDI, D. DUFFRENE and M. M. BURGEOIS, *Lyon Chir.* **57**, 802 (1961).
57. P. MALLET-GUY, C. BOSSER and J. MICHOULIER, *Bull. Soc. int. Chir.* **5–6**, 531 (1963).
58. R. MARX, *Thrombos. Diathes. haemorrh., Stuttg.* Suppl. Vol. II (1958).
59. R. MARX, P. CLEMENTE, E. WERLE and W. APPEL, *Blut* **5**, 367 (1959).
60. R. MARX, *Internist* **6**, 235 (1965).
61. W. MAURER, *Schweiz. med. Wschr.* **94**, 1743 (1964).
62. A. MEYER, *Habilitationsschrift*, Med. Fakultät, Munich (1964).
63. A. MEYER and E. WERLE, in N. HENNING, K. KEINKEL and H. SCHÖN, *Pathogenese, Diagnostik, Klinik und Therapie der Erkrankungen des exokrinen Pankreas*,p. 299. Schattauer, Stuttgart (1964).
64. A. MEYER, Deutscher Chir. Kongress, Munich (1965).
65. D. C. NABSETH, K. APOSTOLOU, B. P. KEKSIS, S. CHEREN and A. HIRSCH, *Fedn Proc.* **23**, 605 (1963).
66. M. NAGEL, Deutscher Chir. Kongress, Munich (1965).
67. W. NAGEL and F. WILLIG, *Klin. Wschr.* **42**, 400 (1964).
68. W. NAGEL, K. P. ROBEL and F. WILLIG, *Klin. Wschr.* **43**, 173 (1965).
69 P. NEMIR, J. HOFERICHTER and D. L. DRABKIN, *Ann. Surg.* **158**, 655 (1963).
70. F. W. NUGENT, M. E. HENDERSON, H. JONASSON, C. F. G. JONES and W. A. ATENDIDO, *Lahey Clin. Bull.* **13**, 167 (1964).
71. S. RIBACOFF, A. HURWITZ, R. KHAFIF and F. SIEGMAN, *Surg. Forum* **12**, 370 (1961).
72. J. W. RYAN, J. G. MOFFAT and A. G. THOMPSON, *Nature, Lond.* **204**, 1212 (1964).
73. J. W. RYAN, J. G. MOFFAT and A. G. THOMPSON, *Archs. Surg.* **91**, 14 (1965).
74. H. SCHÖN and N. HENNING, *Klin. Wschr.* **41**, 201 (1963).
75. H. SCHÖN, K. HEINKEL and N. HENNING, *Klin. Wschr.* **41**, 612 (1963).
76. H. SCHÖN and N. HENNING, *Med. exp., Basel* **8**, 83 (1963).
77. G. SCHÖNBACH, F. X. SAILER, D. STAMM, G. KÜMMEL and H. D. MÖLLER, *Klin. Wschr.* **44**, 652 (1966).
78. K. SCHULTIS and W. RICK, *Verh. dtsch. Ges Inn. Med.* **70**, 824 (1964).
79. F. SCHULTZ, H. KRAUT and N. BHARGAVA, *Naturwissenschaften* **50**, 375 (1963).
80. A. J. SCHUTT, R. SPRAGUE, L. G. BARTHOLOMEW and J. C. CAIN, *J. Lab. clin. Med.* **62**, 1013 (1963).
81. A. J. SCHUTT, K. G. WAKIM, L. G. BARTHOLOMEW, J. C. CAIN and A. H. BAGGENSTOSS, *J. Am. med. Ass.* **191**, 905 (1965).
82. R. B. SMITH, R. C. ORAHOOD, S. L. WANGENSTEEN, G. J. BERAKHA and A. ZAMELIS, *Surgery* **54**, 922 (1963).
83. A. P. THAL, E. E. KOBOLD and M. J. HOLLENBERG, *Am. J. Surg.* **105**, 708 (1963).
84. TRASYLOL Symposium, Grosse Ledder (1965). (Eds. R. GROSS and C. KRONEBERG), Neue Aspekate der Trasylol-Therapie, Schattauer Verlag, Stuttgart (1966).
85. I. TRAUTSCHOLD, Vortrag: Gemeinsame Tagung der Schweiz. und Dtsch. Gesellschaft f. Physiologische Chemie, Zürich (1960).
86. I. TRAUTSCHOLD and E. WERLE, *Hoppe-Seyler's Z. physiol. Chem.* **325**, 48 (1961).
87. I. TRAUTSCHOLD and E. WERLE, in H. U. BERGMEYER, *Methoden der enzymatischen Analyse*, p. 880. Verlag Chemie (1962).
88. I. TRAUTSCHOLD and G. RÜDEL, *Klin. Wschr.* **41**, 297 (1963).
89. I. TRAUTSCHOLD, E. WERLE, H. HAENDLE and H. SEBENING, in N. HENNING, K. HEINKEL and H. SCHÖN, *Pathogenese, Diagnostik, Klinik und Therapie der Erkrankungen des exokrinen Pankreas*, p. 289. Schattauer, Stuttgart (1964).
90. I. TRAUTSCHOLD, *Habilitationsschrift*, Med. Fakultät, Munich (1965).
91. I. TRAUTSCHOLD, H. FRITZ and E. WERLE, (Eds. E. G. ERDÖS, N. BACK and F. SLEUTERI) *Hypotensive Peptides*, p. 221, Thieme, Florence (1965).
92. Y. TSUKIYAMA, S. AOYAMA and S. YOSHIOKA, Ann. Meet. Gastr., Jap Tokyo (1961).
93. Y. TSUKIYAMA, H. FUKUDA, Y. KUBOTA, H. YOSHIMURA, S. YOSHIOKA and M. NAKAYAMA, 48th Ann. Meet. Gastr. Soc. Jap., Tokyo (1962).
94. R. VOGEL, I. TRAUTSCHOLD and E. WERLE, *Natürliche Proteinasen-Inhibitoren.* Thieme, Stuttgart (1966). Academic Press, New York. In press.

95. M. E. WEBSTER and W. R. CLARK, *Am. J. Physiol.* **197**, 406 (1959).
96. E. WERLE, W. GÖTZE and A. KEPPLER, *Biochem. Z.* **289**, 217 (1937).
97. E. WERLE and M. GRUNZ, *Biochem. Z.* **301**, 429 (1939).
98. E. WERLE, L. MAIER and E. RINGELMANN, *Naturwissenschaften* **39**, 328 (1952).
99. E. WERLE, Second Int. Biochem. Congr., Brussels (1956).
100. E. WERLE, Anmerkung in, E. WERLE and W. APPEL, *Z. Naturforsch.* **14b**, 385 (1959).
101. E. WERLE, *Angew. Chem.* **73**, 689 (1961).
102. E. WERLE and I. TRAUTSCHOLD, *Münchn. med. Wschr.* **103**, 773 (1961).
103. E. WERLE, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **245**, 254 (1963).
104. E. WERLE, *Hoppe-Seyler's Z. physiol. Chem.* **338**, 228 (1964).
105. E. WERLE and I. TRAUTSCHOLD, Sixth Ann. Meet. Anglo-German med. Soc., Manchester (1965).
106. H. ZOEPFFEL, *Klin. Wschr.* **1**, 123 (1922).
107. H. ZOEPFFEL, *Dt. Z. Chir.* **175**, 301 (1922).