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Kallikreins (Kininogenases)—A Group of Serine Proteases with Bioregulatory Actions*

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I. Introduction

THE kallikreins (E.C. 3.4.21.8) are a group of serine proteases with specific and limited proteolytic actions. They release a peptide, kinin, from a substrate, kininogen, that is present in lymph, plasma, and interstitial fluid in mammals. Therefore, the kallikreins are also called kininogenases. They have marked actions on blood vessels and smooth muscles in vivo that are indirect, i.e., due to the release of the pharmacologically active peptide, kinin. It has become clear in recent years, however, that in some instances at least, the physiologically significant proteolytic actions of these enzymes are unrelated to the release of kinin (176, 177, 207, 208, 239), a view long held by the reviewer (23, 211).

* This review is dedicated to the memory of my colleague and friend, Professor Eugen Werle (1902–1975), distinguished scholar and a pioneer of the kallikrein-kinin system and its inhibitors.

Recent analyses of amino acid sequences and three-dimensional structures indicate structural homology and a common catalytic mechanism for many of these enzymes and suggest a common molecular ancestry for the kallikreins and other serine proteases (176, 177, 207, 208, 239).

In this review, an attempt is made to present a diffuse and historically complex literature in a succinct way by selecting areas of relative significance. The subject of the kallikrein-kinin system has grown so in the last 10 years that this survey is restricted to the kallikreins, other kininogenases, and related proteases. The kinins, the peptides that they release, which also occur free in nature, are not described here. Reference is made to them, however, where the context requires it.

II. Historical Data

In order to clarify the current terminology and to integrate the diffuse but significant findings over a period of 50 years, it is necessary to survey briefly the historical development of the kallikreins and related substances. The historically minded reader is referred to the following monographs and reviews (72, 202, 213, 214, 249, 260, 270). For details of the broad subject, many reviews and symposia may be consulted (72, 94, 171, 179, 195, 202, 203, 213, 214, 242–249, 251, 252, 260, 261, 270).

The discovery of kallikrein can be regarded as having begun in 1926 to 1928, when Frey (69) and Frey and Kraut (70) found that human urine contained a thermolabile, nondialyzable substance that, on intravenous injection, produced a prolonged arterial hypotension in the dog. Since Frey and coworkers regarded this substance in urine as an excreted hormone that affected the blood vessels and circulation, they called it a *Kreislaufhormon* (70, 126). Searching for the organ in which this presumed hormone originated, Werle and coworkers found a similar substance in pancreas (127) and also in blood (129, 267). In both instances the substance was present in an inactive but readily activatable form. They assumed, therefore, that it originated in the pancreas and named it kallikrein, from *Kallikreas*, the Greek word for pancreas (127). This name was perhaps an unfortunate choice, since it is now known that although the different kallikreins are serine proteases with a significant chemical similarity, they are neither identical nor do they originate from a parent molecule (178, 268, 270). Urinary kallikrein is now thought to originate largely from the kidney (180, 181).

Beginning in 1930, Werle and his colleagues soon discovered most of the known components of the kallikrein-kinin system. Thus, as early as 1930 (128, 270), they described the basic kallikrein inhibitor in extracts of bovine pancreas. This was one of the first descriptions of a natural protease inhibitor. In less than 10 years they characterized the kallikreins in pancreas, urine, blood, and salivary glands. They also briefly described others in intestine, lung, brain, and nerve (71, 214, 268, 270). In 1937 (274), they demonstrated that the kallikreins released a substance enzymically from an inactive precursor in plasma and that the released substance contracted isolated smooth muscle preparations. They named it substance DK, from "darmkontrahierende Substanz." In 1939 (275), Werle and Grunz showed that substance DK lowered the arterial blood pressure and that cysteine potentiated its action in vitro and in vivo by preventing its enzymic degradation. This demonstration of the release of substance DK by kallikrein preceded by two years the analogous demonstration that renin owed its hypertensive action to the release of a pressor peptide from a substrate in plasma (29, 189). In 1948 (272), Werle and Berek recommended that substance DK be renamed kallidin, and that its precursor, the substrate for kallikrein, be called kallidinogen.

In 1949 (204), Rocha e Silva et al. described the release of an active peptide by trypsin and certain snake venoms from a substrate in the globulin fraction of plasma. They named the active peptide bradykinin. It is now clear that the trypsin- or snake venom-bradykininogen-bradykinin system is very similar to the kallikrein-kallidinogen-kallidin system.

In 1954 (218), Schachter and Thain introduced the term kinin because it appeared to them, from a direct comparison, that kallidin and bradykinin belonged to a group of closely related peptides that occurred naturally in wasp, hornet, and other venoms, or were released from a plasma substrate by kallikreins, trypsin, snake venom, and possibly other proteases (100, 209, 210). In 1961, Schachter et al. (23) introduced the term *kininogenase* as a generic term for all enzymes that release a kinin and the term *kininogen* for the substrate. Hence, the name kininogenase includes kallikreins, trypsin, snake venom, and other enzymes that yield a kinin by limited proteolysis of kininogen.

With the benefit of hindsight, it now appears that the early German investigations, with the blood pressure of the dog as an assay system for *kallikrein*, uncovered a group of serine proteases that had in common the limited proteolytic property of releasing kinin from dog kininogen, thus producing a hypotensive effect. Related serine proteases with a different specificity were not detected. Werle and his colleagues (279), in fact, failed to detect the kininogenase activity of trypsin, because trypsin, when compared to the glandular kallikreins, is relatively ineffective as a hypotensive agent in the dog, presumably because the protease inhibitors in dog plasma are very effective as trypsin inhibitors.

In this review, the term kallikrein is retained for those enzymes described many years ago in urine, pancreas, plasma, and salivary glands. Also, since renal kallikrein is now regarded as the major source of the kininogenase in urine, it, too, is called a kallikrein.

III. Kallikreins and Other Kininogenases

A. General Properties

Results since 1950 (see 72, 127, 207, 208, 213, 214, 261) have altered significantly some of the earlier views about kallikreins. Thus, it is now clear that pancreatic kallikrein

is not the origin of plasma kallikrein, nor is either one the source of urinary kallikrein. However, these kallikreins and the newer kininogenases are sufficiently similar chemically, in some instances almost identical (74), to be classified as a generic group. Like other enzymes they occur in multiple forms. This has been found for urinary (74, 180), submandibular (56, 180), and pancreatic kallikreins (61).

All kallikreins and other kininogenases studied are serine proteases and have active sites and catalytic mechanisms very similar to those of enzymes like trypsin, chymotrypsin, elastase, thrombin, plasmin, the $C\bar{1}$ esterase component of complement, and other serine proteases (130, 176, 195, 239). Histidine, serine, and aspartic acid are the amino acids that form the significant spatial configurations of the active sites in all these enzymes. The kallikreins are glycoproteins with molecular weights mostly between 25,000 and 40,000 and, except for plasma kallikrein, are highly specific in their substrate requirements, the different kallikreins showing some differences (62, 195, 214, 240). Details of configuration of substrate may be significant, e.g., the tertiary structure of kininogen appears to increase the susceptibility of the peptide bond Met-Lys to pancreatic kallikrein (109).

B. Measurement

Until recently, kallikreins and other kininogenases were measured by classic bioassay techniques and by one chemical method, viz., the hydrolysis of various synthetic esters. Recently new chemical methods have been introduced and they are discussed below.

Bioassay is still performed by recording the hypotensive activity of the substance on the dog's blood pressure and comparing it with some standardized preparation of kallikrein or by measuring the ability of a substance to release kinin from a suitable substrate and measuring the kinin released with an isolated smooth muscle preparation such as the guinea-pig ileum or rat uterus. The advantage of bioassays over chemical tests is that they measure directly a relevant and specific biological property of the kininogenase. However, in addition to being imprecise, bioassays have pitfalls insofar as the potency of the enzyme as a kininogenase depends on the specific mammalian kininogen on which it is acting. For example, cat salivary kallikrein does not release kinin from horse plasma but horse salivary kallikrein does so readily (24). Also, guinea-pig coagulating gland kininogenase (CGK) releases kinin from plasma of the guinea pig, dog, cat, ox, rabbit, and man, but fails to release kinin from rat plasma, although trypsin and various kallikreins do so readily (23, 212).

All killikreins and kininogenases studied thus far hydrolyze synthetic amino acid esters, e.g., N-benzoyl-L-arginine methylester (BAME), toluolsulfonyl-L-arginine methylester (TAME), and N-acetyl-L-tyrosine-ethylester (ATEE). Other synthetic substrates for trypsin such as benzoyl-arginine-naphthylamide (BANA) and benzoyl-arginine-*p*-nitroanilide (BAPA) are hydrolyzed by plasma kallikrein but not by salivary or urinary kallikreins (72, 90, 256). Pisano et al. (15, 107) have developed a radiochemical esterolytic assay for kallikrein with [^{3}H]-TAME labelled in the methyl group of the alcohol moiety of the synthetic ester substrate. They describe this method as rapid, simple, and sensitive (107).

Synthetic peptide *chromogenic* substrates have been introduced recently to measure kallikreins and other proteases. These substances are *p*-nitroanilides of tri- and tetrapeptides with various groups substituted at the terminal amino group. The chromogenic substrate itself is colourless but the *p*-nitroaniline liberated on proteolysis is a coloured compound that is measurable. These chromogenic substrates are now being used widely (3, 4, 37, 38, 122). Synthetic peptide *fluorogenic* substrates have also been introduced with alleged greater sensitivity than the chromogenic ones (165). The chromogenic and fluorogenic substrate methods, like those with synthetic ester substrates, have problems of sensitivity and specificity, and in each case, experience must also be acquired in order to deal with interfering factors.

Finally, radioimmunoassay procedures have been developed (63, 64, 66, 142). These have the property of remarkable sensitivity, detecting concentrations of kallikreins in concentrations of picograms per ml. There are, however, problems with the complexity of the method and also with its limited specificity, the latter resulting, for example, from the immunological crossreactivity of salivary, pancreatic, and urinary kallikrein in the same species (74, 197). In fact, these glandular kallikreins, even from different mammals, may crossreact. (219).

C. Chemistry

A recent comparison of porcine pancreatic, submandibular, and urinary kallikreins showed that these three enzymes have almost identical amino acid compositions and have partial sequences in common (60, 74). They are crossreactive immunologically and have similar dissociation constants with various protease inhibitors.

Tschesche et al. (258) have established the complete amino acid sequence of porcine pancreatic kallikrein B. They conclude that the overall homology with trypsin consists of 37% of identical residues in corresponding position and 51% of chemically similar residues. They also emphasize the striking similarity of kallikrein and trypsin in that 57% of the residues mediating contact between trypsin and the polyvalent kallikrein-trypsin inhibitor, as shown by Huber et al. (104), are also present in kallikrein.

Porcine pancreatic kallikrein B contains about 11.5% carbohydrate, which is about twice that of the porcine kallikrein A. However, both kallikreins were found to have identical amino acid compositions with 232 residues each (MW 25,600). They both consist of two peptide chains interlocked by disulphide bridges (104). The porcine pancreatic kallikreins have been studied the most. The amino acid compositions of many other kallikreins are, however, also known (see 79, 163, 244, 246, 247).

It has been known from the early observations that some kallikreins exist in a preactive form, whereas others are in tissues in an active form (72, 214). Plasma kallikrein and pancreatic kallikrein seem to exist entirely in a preactive although readily activatable form. Plasma kallikrein, of which there are at least three similar forms in blood, may differ significantly from the other kallikreins. It has a much higher molecular weight, 100,000 or more, and is less specific in its substrate requirements, which probably accounts for its broader susceptibility to protease inhibitors (9, 43, 80, 97, 195, 214, 253). The molecular weight of human plasma prekallikrein and that of hog pancreatic prekallikrein undergo no significant change on activation, indicating that the peptide of activation is small (279). In contrast, rabbit plasma prekallikrein, when activated, undergoes a change in molecular weight from 100,000 to 88,000 as calculated from sodium dodecyl phosphate (SDS) electrophoresis measurements (41). Enzymic activators of the prekallikreins are related serine proteases such as trypsin (273), Hageman factor (114), or possibly kallikrein itself (39).

IV. Kininogen

When it was realized in 1937 (274) that the pharmacodynamic actions of the kallikreins in vivo might be due to the proteolytic release of substance DK, it became important to prepare the substrate, now called kininogen, free of prekallikrein, of circulating inhibitors of kallikrein, and of kininases. Werle (274) achieved this by simply heating serum or plasma for 1 to 3 hours at 56°C to 60°C. Diniz and de Carvalho (52) later developed a simple method for the estimation of kininogen in plasma that was used for many years. Early studies indicated that plasma contained more than one kininogen and that different kininogenases had preferential specificities for these substrates (72, 118, 195, 214). Kininogens are acidic glycoproteins and, in general, plasma contains more than one. They are designated as high- and low-molecular weight forms, viz., HMW and LMW kininogens respectively. The HMW and LMW forms of bovine origin have been studied extensively (93, 109, 118, 119, 124) and have molecular weights of 76,000 and 48,000 respectively. In *bovine* kininogen the kinin moiety is in the internal portion of the chain in the HMW kininogen, whereas in the LMW form it is in the terminal carboxyl end (118).

Various molecular forms of *human* kininogen have also been isolated (87, 89, 92, 174, 193, 194, 230). Nakayasu and Nagasawa (174) have determined the amino acid composition of a human kininogen. It is an acidic glycoprotein with a molecular weight of 120,000 and has considerable similarity to bovine kininogen. Like bovine HMW kininogen, the human kininogen contains a histidine-rich peptide (HRP), but, unlike bovine kininogen (119), the HRP sequence is not released when human plasma kallikrein releases kinin from human kininogen. In the latter instance, the HRP sequence remains bound to the light chain of kinin-free kininogen. The histidine-rich peptide (HRP) sequence of kininogen is discussed later in section VI, PLASMA KALLIKREIN-KININOGEN-BLOOD COAGULATION.

Recent studies on inherited deficiencies of human HMW kininogen reveal a function for it other than as a substrate from which kallikrein releases kinin. Together with prekallikrein (Fletcher factor) it is required for the optimal activation of Hageman and possibly other coagulation factors (118, 141, 198). This topic is discussed more fully in section V.

Bovine and human HMW kininogen and other plasma proteins are degraded in a "nonspecific" way by proteases from polymorphonuclear (PMN) leukocytes. Granulocyte elastase in particular is a powerful degrader of HMW kininogen without releasing kinin (183, 184). This action is so effective and the amount of elastase available is so great that Dittman et al. (53) suggest that destruction of clot-promoting activity of HMW kininogen and of other significant proteins could be aetiological factors in the severe bleeding tendency in septicemia and acute leukemia.

V. Naturally Occurring Inhibitors of Kallikrein

The kallikreins, like the well-known serine proteases such as trypsin and thrombin, are susceptible in varying degree to the many protease inhibitors of animal and plant origin. The vast subject of protease inhibitors is covered in extensive reviews (72, 163, 243, 247, 249, 250, 260). This review confines itself to recent developments, in particular to the naturally occurring kallikrein inhibitors other than those of microbial or plant origin.

A. Polyvalent Kallikrein-Trypsin Inhibitor (Kunitz) in Bovine Organs

There have been major developments in our knowledge of the polyvalent protease inhibitor (Kunitz); its chemical structure and cellular location have recently been determined. Beginning in 1930, Kraut, Frey, Werle, and their colleagues (128) studied a *kallikrein* inhibitor extracted from bovine organs, particularly from the parotid gland and lymph glands, but also from liver, pancreas, and spleen (127, 160, 269, 271). Commercial preparations (Trasylol, Zymofren, Iniprol, Contrykal) are prepared either from bovine lung or parotid gland. A *trypsin* inhibitor, also prepared from bovine pancreas, was described independently by Kunitz and Nothrop in 1936 (131). In 1965 to 1966, these independent studies merged when the amino acid sequences for the bovine kallikrein and trypsin inhibitors respectively were independently determined by Anderer and Hörnle (5, 6) and by Kassell et al. (116, 117). The kallikrein inhibitor and the trypsin inhibitor of ruminants were found to be identical, each with 58 residues, three S-S bridges, and a molecular weight of 6,511. In 1970 its tertiary structure was reported by Huber et al. (102, 103) and later further defined (49).

In 1979, almost 50 years after its discovery as an inhibitor of kallikrein, Fritz et al. (75) localized the

polyvalent kallikrein-trypsin inhibitor (Trasylol, Kunitz) in the mast cells of bovine lung, liver, pancreas, and parotid gland by using light and electron microscopic immunocytochemical techniques. This cellular localization explains its long-known presence in *many* tissues of ruminants (72, 75, 214, 260). It would appear probable that this inhibitor, or a species specific variant of it, will now be found in mast cells of man and other mammals as well as in ruminants.

In contrast to the *tissue* or Kunitz inhibitor, the *specific* or *secretory* Kazal trypsin inhibitor has been found in many mammals, including ruminants and man, but only in the pancreas (see 163, 179, 249, 260). The Kazal inhibitor, unlike the Kunitz inhibitor, is secreted into the pancreatic juice with the digestive enzymes and like them is located in the acinar zymogen granules (65, 77, 84). It is also far more specific as an inhibitor, favouring trypsin.

The localization of the Kunitz inhibitor in mast cells suggests a significance for it quite different from that of the Kazal inhibitor. The chemical structures of both have been established and they are not homologous molecules but rather two distinct families of protease inhibitors (65). Fink and Greene (65) have developed radioimmunoassays for both these enzymes that should be of value in determining their physiological and other significance.

B. Kallikrein Inhibitors in Plasma

The first observations on the inhibition of kallikrein by human and other mammalian plasmas or sera were made by Kraut et al. (126) in 1928 and the subject was investigated more thoroughly by Werle (266) in 1934. It was initially assumed that the "inactive" (preactive) kallikrein in normal plasma was a complex of kallikrein and a circulating inactivator (see 71). Plasma prekallikrein, however, is a precursor, activatable by trypsin and Hageman factor, and is thus analogous to prothrombin, trypsinogen, and other proenzymes (41, 273). It is now apparent that the kallikrein inhibitors of plasma are large molecular weight proteins with the ability to combine with the catalytic sites of various endogenous proteases. They comprise about 10% of the total plasma proteins (98). Four kallikrein inhibitors of varying potency have thus far been identified in plasma, viz., $C\bar{1}$ esterase inhibitor, α_2-macroglobulin, α_1-antitrypsin, and antithrombin III (9, 78, 81, 132, 154, 155, 257) (fig. 1).

Inherited specific deficiencies of C1 esterase inhibitor (54, 133), α_1-antitrypsin (164, 280), and antithrombin III (145) have all been identified clinically. This is now also true for other components of the kallikrein system such as prekallikrein (Fletcher factor) (265, 283), kininogen (42, 206, 284), and plasma kininase I (237). These deficiencies should help establish the significance of individual components of the human kallikrein-kininogen-kinin system.

The inhibitors have also been of some, although limited, value in establishing the significance of kallikreins.

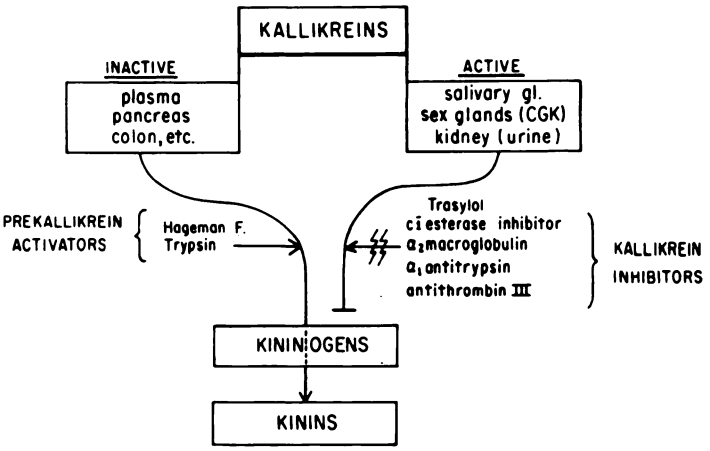

FIG. 1. Diagram of main components of kallikrein-kininogen-kinin system. It is simplified for conciseness. For example, Hageman factor normally activates plasma prekallikrein only. Again, the different kallikrein inhibitors shown on the right of the diagram inhibit in varying degree not only the active kallikreins, but also the *activated* kallikreins of plasma, pancreas, colon, etc., shown on the left side of the diagram. See text for details.

Their limitation in this respect is that because of the close similarity in the structures of the catalytic sites of these enzymes, the inhibition is not specific to a single protease with a specific substrate, but involves, in varying degree, a large population of related serine proteases.

VI. Plasma Kallikrein-Kininogen-Blood Coagulation

Plasma kallikrein, once thought to be the circulating form of pancreatic kallikrein, is now known to differ most from the other kallikreins. It has a far higher molecular weight, is less specific in its substrate requirements, in which it resembles trypsin, and, like the latter, it releases bradykinin rather than lys-bradykinin from kininogen (41, 43, 80, 97, 253).

Recent studies on inherited plasma prekallikrein deficiency have revealed a significant role for the enzyme in blood coagulation unrelated to the release of kinin from kininogen. In 1973, Wuepper (283) discovered an inherited deficiency of plasma prekallikrein, which had been named Fletcher factor after the patient in whom the condition was first described by Hathaway et al. (96) in 1965. Plasma deficient in Fletcher factor, like Hageman factor (HF)-deficient plasma, fails to generate kinin on contact with kaolin or glass and shows a prolongation of thromboplastin generation in vitro. Patients with Fletcher or HF deficiency, however, do not have either coagulation or other haemostatic problems (96, 283).

Figure 2 illustrates some of the probable relationships between the kallikrein-kinin system in plasma and the intrinsic blood coagulation system. In this scheme, HF (factor XII) occupies a central role, linking both systems, and the scheme includes a new property of plasma kallikrein, viz., its ability to activate pre-HF. It thus provides a feedback mechanism for sustained HF formation (39,

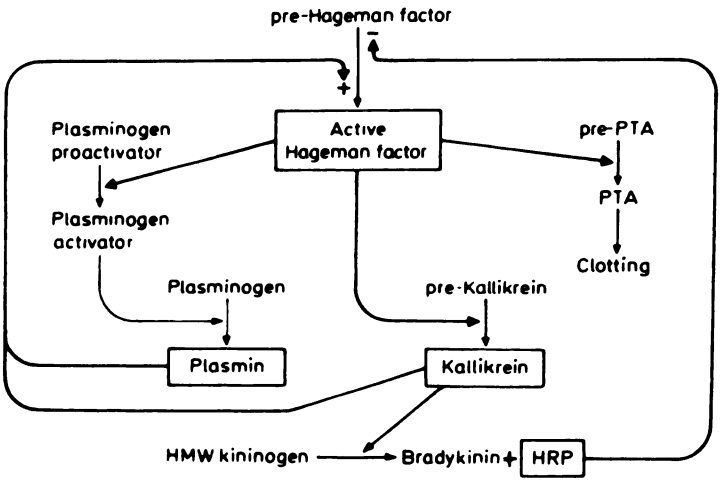

FIG. 2. Diagram showing interrelationship between plasma kallikrein and blood coagulating system. [From Katori et al. (119)]. See figure 3 and text for additional procoagulant role of high molecular weight (HMW)-kininogen and other details.

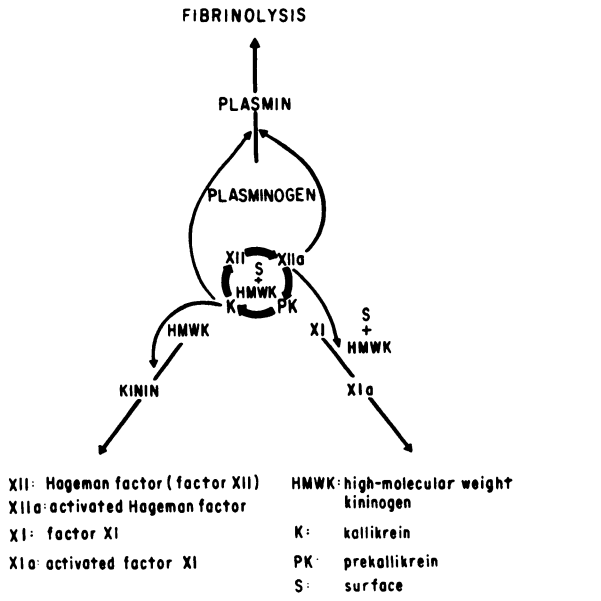

FIG. 3. Diagram showing surface-mediated reactions in blood coagulation system in man and possible sites of participation of plasma prekallikrein, kallikrein, and high molecular weight kininogen. Details in text. [Modified from Ogston and Bennett (182)].

40, 114, 141, 198, 265) since HF itself activates prekallikrein and PTA (33, 115, 138, 159).

Individuals with Fletcher factor deficiency have in vitro defects in coagulation (intrinsic system), in fibrinolysis, in kinin generation, and in the chemotactic properties of their plasmas. All these defects are corrected by the addition of prekallikrein, which activates pre-HF as shown in figure 2. The figure also shows that plasminogen, like prekallikrein, is activated by HF or by fragments of HF derived during its activation (115). This explains older observations of fibrinolytic defects in HF-deficient plasma (106). Figure 2 shows that plasmin, like kallikrein, is a positive feedback agent for HF-dependent pathways (115).

In addition to prekallikrein deficiency, there are now known inherited deficiencies of HMW kininogen that indicate that the latter molecule is involved in the intrinsic blood clotting system independently of it being a kinin precursor. This deficiency is also named after families in which it is absent, e.g., Flaujeac, Williams, or Fitzgerald (43, 118, 141, 198, 206, 284). These patients also have little or no haemostatic defect but, as in Fletcher factor deficiency, their plasmas show abnormalities in surface- or contact-mediated coagulation, fibrinolytic and kinin-generating pathways, and have a prolonged partial thromboplastin time. It is not clear, however, how, or exactly where in the intrinsic clotting system, HMW kininogen acts. Figure 3 indicates the sites at which HMW kininogen is thought to act to enhance the activation of factors XII, prekallikrein, factor XI, and plasminogen activator. For a relatively concise analysis of this complex subject, the article by Ogston and Bennett (182) may be consulted.

Figure 2 also illustrates a recent suggestion by Suzuki and coworkers (93, 119) that there may also be a negative feedback on the intrinsic clotting system. They have shown that bovine plasma kallikrein releases, in addition to bradykinin, two peptide fragments from pure bovine HMW kininogen, one of which is designated as HRP. The basic amino acids, histidine, glycine, and lysine, constitute about 70% of the molecule of HRP. It is a potent inhibitor of the activation of pre-HF. Suzuki and coworkers (119) suggest, therefore, that HRP, in contrast to kallikrein and plasmin, acts as a negative feedback agent on pre-HF, an action appropriate for the final product of a physiologically significant "cascade" system (fig. 2). However, Nakayasu and Nagasawa (174), by using purified *human* plasma kallikrein and kininogen, found that although bovine and human HMW kininogen were similar in composition, the corresponding HRP sequence in the latter was not released by human plasma kallikrein but remained bound to the light chain of kinin-free protein.

A hereditary deficiency in one of the circulating kallikrein inhibitors, viz., the $C\bar{1}$ esterase inhibitor, occurs in hereditary angioedema (54, 133). Its absence results in bouts of spreading subcutaneous, laryngeal, or other edema, probably due to the local release of kinin.

Other inherited deficiencies have been described recently in the plasma kallikrein-kinin system. One of these is characterized by episodes of excessive orthostatic falls in pulse pressure and cutaneous vasodilatation. This condition is thought to be caused by a hereditary deficiency of kininase I and has been described as "hyperbradykininism, a new orthostatic syndrome" (237). A case of auto-DNA sensitization associated with episodes of painful cutaneous areas, edema, and ecchymotic lesions has also been attributed to hyperbradykininism, thought to result from kininase deficiency (134).

Finally, observations with purified plasma kallikrein and also with plasminogen activator indicate that both are chemotactic for human leukocytes (115) and suggest functional links between blood clotting, kallikrein and related serine proteases, and inflammation.

VII. Renal Kallikrein-Hypertension-Prostaglandins

A. Renal and Urinary Kallikrein

It was first suggested by Werle and Vogel (278) that urinary kallikrein originated in the kidney tubules. A tubular localization for renal kallikrein has since been supported by different studies (12, 35, 188). The evidence indicates that renal kallikrein enters the urine from renal distal tubular cells (223, 224). The possibility also exists that some renal kallikrein enters the extracellular space (48, 201).

Nustad et al. (180, 181) have shown that the urine and kidney of the rat each contain four related kallikreins. Those in urine were indistinguishable, however, from those in the kidney. Further, the same four radioactive kallikreins were isolated from rat kidney slices incubated with [^{3}H]-L-leucine, indicating that the kidney is the site of their synthesis (181). Nonetheless, recent experiments with sensitive radioimmunoassays for kallikrein have led Fink et al. (64) to conclude that glandular kallikreins of pancreatic and submandibular gland may be excreted by the kidney and that measurements of kallikrein in urine may include some of these as well.

B. Renal Kallikrein and Hypertension

In 1964 (10, 264), it was shown that the injection of bradykinin into the renal artery produced a diuresis and an increased excretion of sodium from the injected kidney. Kallikreins and kinins are, therefore, diuretic and natriuretic agents.

As early as 1934, in an isolated study, Elliot and Nuzum (58) reported a decreased urinary excretion of kallikrein in patients with hypertension. About 40 years later extensive studies with modern methods of measuring kallikrein and its excretion began. The excretion of kallikrein was confirmed to be subnormal in patients with essential or renovascular (stenosis) hypertension (146, 147, 149–151, 241, 248) and in rats made hypertensive via renal stenosis (45, 120, 241, 248). In contrast, it was found to be increased in primary aldosteronism, in patients and animals receiving sodium-retaining steroids, and in patients with phaeochromocytoma (1, 146, 147, 241, 248). Aldosterone appears to regulate kallikrein secretion in normal subjects, too. They show increased kallikrein excretion in response to a low sodium intake, to a high potassium intake, or to administration of the synthetic mineralocorticoid, fludrocortisone. Correspondingly, kallikrein excretion falls after administration of the antagonist spironolactone (146, 241, 248). Margolius et al. (148) found that incubated suspensions of rat renal cortical cells secreted kallikrein into the medium. This secretion in vitro was increased by aldosterone and decreased by spironolactone. The authors speculated from their observations on whether renal kallikrein might be an aldosterone-induced protein.

Recent details and precise information about the actions and behaviour of renal kallikrein indicate a physiological role for this enzyme and its possible involvement in hypertension and other pathological states. Specific conclusions, however, although possibly not far off, remain elusive. Margolius (147) critically assesses this problem in a recent article appropriately entitled, "Kallikrein, kinins, and the kidney: What's going on in there?"

C. Kallikreins and Prostaglandins

McGiff et al. (158, 255) first reported that kinins activate the prostaglandin-synthesizing system in the kidney. Nasjletti and Colina-Chourio (175) came to the same conclusion from experiments on the isolated perfused rabbit kidney. Further, they also showed that administration of the kallikrein inhibitor, Trasylol, decreased the urinary excretion of prostaglandin in the rat. They conclude that, "a coupling of kinins and prostaglandins intrarenally may be directed towards the facilitation of salt and water excretion" (175).

There is also some clinical evidence that supports a link between kallikrein and prostaglandin, viz., in Bartter's syndrome. This syndrome, first described in 1962 by Bartter et al. (14), is characterized by renal juxtaglomerular hyperplasia, hyperreninemia, hyperaldosteronism, and abnormalities in renal tubular absorption of sodium, but with a normal blood pressure. A significant fact discovered recently about this syndrome is that the increased production of prostaglandins by the kidney mediates the hyperreninemia and hyperaldosteronism that characterize the syndrome (82). Further, Halushka et al. (91) found that, along with increased prostaglandin excretion of 3 and 10 times normal, two cases of Bartter's syndrome also had kallikrein excretions of 5 and 10 times normal respectively. Also, the administration of indomethacin, a prostaglandin synthetase inhibitor, corrected most of the abnormalities relating to prostaglandin, renin, and related factors. They conclude that their clinical observations support the view of a link between the renal kallikrein-kinin system and renal prostaglandins.

Although the specific physiological significance of all these observations remains uncertain, there is much to suggest that prostaglandins mediate or modulate the actions of kinin that is released intrarenally.

D. Renal Kallikrein and Renin

Despite their complexity, the facts suggest that renal kallikrein, like renin, may be of significance in regulating electrolyte and water balance. The report that injection of antibody against bradykinin inhibits natriuresis induced by infusion of isotonic sodium chloride (152) supports this hypothesis.

The observation of Werle et al. (276) that the kininogen concentration of plasma rises to a mean value 3.5 times normal after bilateral nephrectomy suggests that there is a constant consumption of plasma kininogen by renal kallikrein. A similar increase in the concentration of plasma angiotensinogen occurs after nephrectomy (25).

Sealey et al. (225, 226) have raised the question of a possible significant link between kidney kallikrein and renin by showing that urinary kallikrein is a potent activator of plasma prorenin in vitro. They suggest that the location of kallikrein in distal tubules and macula densa cells may make it possible for it somehow to "trigger" the renin located in the adjacent juxtaglomerular cells.

VIII. Kallikreins and the Reproductive System

A. Coagulating Gland Kininogenase

The presence of powerful proteolytic enzymes in seminal plasma has long been known (143). Extensive studies were conducted as long as 30 to 40 years ago on fibrinolytic and fibrinogenolytic enzymes (105) in semen, as well as on other enzymes resembling trypsin, pepsin, and other proteases (139). In recent years there has been much progress because of developments in enzyme and protein chemistry. Excellent reviews of proteolytic enzymes in the reproductive tract of mammals are available (171, 286).

The first evidence of a kallikrein-like enzyme in reproductive organs was presented for the accessory sex glands of the guinea pig by the reviewer and his colleagues in 1961 (23, 212). A potent kininogenase—the term kininogenase was introduced at this time for this enzyme—was found in large amounts, particularly in the coagulating gland but also in the prostate gland of the guinea pig. This enzyme released kinin from the plasma of all mammals tested except the rat. In contrast, the coagulating gland of the rat and the prostate gland of rat, rabbit, dog, and man did not release a kinin from plasma. The name of the enzyme was later abbreviated to CGK (coagulating gland kininogenase) (169) and the kinin released was shown to be either bradykinin or lys-bradykinin (169, 170). The amino acid composition of CGK has been reported (168, 170). Its cellular and subcellular localization has been studied by centrifugation of tissue homogenates (13) and by immunocytochemical (217) techniques. Submandibular gland kallikrein of the guinea pig crossreacts with antibody to CGK, although the immunoreactivity is considerably less than with CGK itself (217).

Recently nerve growth factor (NGF) has been reported to occur in large amounts in the prostate gland of the guinea pig by Harper et al. (95). The prostate gland in the guinea pig, along with the coagulating gland (part of the prostate complex in rodents), is also one of the richest sources of kininogenase or kallikrein (CGK) (23, 168, 169, 212, 217). This underlines the close coexistence, probably in the same cells, of high concentrations of both NGF and kallikrein in three different locations, viz., snake venoms, mouse submandibular gland and its secretion, and guinea-pig prostate gland and its secretion.

B. Acrosin

A sperm acrosomal protease that resembles trypsin has been purified and studied intensively in recent years (76, 221, 232, 286). It was named *acrosin* in 1971 by Zaneveld et al. (286). It is a glycoprotein and the amino acid composition has been determined for acrosin from several mammalian species (221, 232). Stambaugh and Smith (232) found a marked similarity in amino acid composition between rabbit acrosin and human trypsin. This was not found to be the case, however, when boar acrosin and trypsin were compared (22). Nonetheless, according to Fritz et al. (73, 190), boar acrosin is very similar to trypsin in substrate specificity, in reactions with inhibitors, and in kinin-releasing potency.

There is some uncertainty as to how much of the enzyme in the acrosome occurs as proacrosin (73, 160, 161, 171). It is proposed that the main functions of acrosin may be to digest a path through the zona pellucida of the ovum while increasing sperm motility and thus facilitating sperm penetration of the digested zona. Müller and Fritz (172) suggest that acrosin acts in a membrane-associated state. They argue that while the amount of acrosin per boar spermatozoon is very low, approximately 10^{-18} moles, the local concentration on the acrosin membrane is considerable, 10^{-2} M. This could explain why the spermatozoon is able to digest a very narrow channel through the zona pellucida. According to Morton (171), the role of acrosin in sperm penetration of the zona pellucida is compelling but not conclusive. He suggests that experiments involving highly selective inhibition of acrosin would help to answer the question.

C. Inhibitors of Acrosin

Acrosin is sensitive in varying degree to the many trypsin inhibitors. Inhibitors of acrosin and other proteases are abundant in accessory sex glands, their secretions, and in seminal plasma (171, 247). In general, these genital tract inhibitors have low molecular weights of 6,000 to 12,000 and are characterized by their stability at low pH values of about 2. They are present in secretions of the genital tracts of both sexes. Reported failures to detect acrosin in sperm of some mammals, e.g., the mouse, may be due to the difficulty of separating it from the surrounding inhibitors (67).

The possible value of acrosin inhibition for the purpose of contraception is obvious. Already in 1969, Stambaugh et al. (231), reported the inhibition of fertilization of rabbit ova in vitro by trypsin inhibitors. They concluded that their experiments provided "evidence that the trypsin-like enzyme is the essential enzyme for penetration of the zona pellucida of the ovum." Recent studies have

been carried out in vivo but difficulties have arisen for various reasons, amongst which is the lack of specificity of good inhibitors. The problem is discussed in a review by Morton (171).

D. Possible Role of Kallikreins in Reproductive Physiology

Kallikrein has been reported not only to increase sperm motility in vitro (135, 235), but also to increase spermatogenesis after its injection into rats (254). Treatment by injections of kallikrein has been reported to produce positive results in treatment of male infertility (108). More work is required to clarify these problems.

Changes in components of the plasma kallikrein system have been reported to occur in women and animals during the estrous and menstrual cycles, pregnancy, and parturition (156, 157, 268, 277). The plasma kininogen concentrations rise 2- to 4-fold during pregnancy and fall to normal after parturition. It has been suggested that the kallikrein-kinin system may be involved in ovulation as well as in parturition because of the cyclic variations in kininogen concentrations (157). Melmon et al. (162) suggested that plasma prekallikrein is activated at birth and that the released kinin mediates the neonatal vascular changes, viz., constriction of the umbilical vessels and ductus arteriosus and dilatation of the pulmonary vasculature. At present all these suggestions are speculative.

IX. Subcellular Localization of Kallikrein

A. Introductory Data

In 1960, Forell (68) suggested that kallikrein was located with the other digestive enzymes in the pancreas because the kallikrein and digestive enzyme content of the pancreas in rats decreased in parallel after ligation of the pancreatic duct. Furthermore, there was no reduction in kallikrein content when the β-cells of the islets of Langerhans were destroyed by treatment with alloxan. In 1968, Werle et al. (72) showed that prekallikrein, trypsinogen, and the secretory trypsin inhibitor (Kazal) were secreted in parallel from the pancreas of the dog. They concluded that "prekallikrein is stored together with trypsinogen and the specific trypsin inhibitor in the zymogen granules of the pancreas and is secreted together with them."

Recently a *direct* approach to enzyme localization with immunocytochemical and related techniques has been used to localize kallikreins and kininogenases in the salivary glands, pancreas, kidney, coagulating gland, and sperm. These studies are described below.

B. Salivary Glands

In 1971, Ekfors and Hopsu-Havu (56) described the localization of "trypsin-like esteropeptidases" in the granular ducts (or tubules) of the mouse submandibular gland by an immunofluorescence technique. They found no immunoreactivity in the acini or elsewhere. The authors considered kallikrein to belong to this group of enzymes. These findings were in contrast to those of others who concluded that kallikrein is located in the zymogen granules of the acinar cells (17–19, 21). More recent immunocytochemical and related techniques have localized kallikrein largely, possibly exclusively, in the duct cells of the salivary glands of the rat (28, 187), cat (11, 99, 144), guinea pig (153, 217), pig (51), mouse (227), dog, and man (153). The results (2, 17–22), from which it was concluded that kallikrein is localized in acinar "zymogen" granules in salivary glands of many mammals, were probably due to difficulties with earlier methods. It is also possible that there may be some exceptions to an exclusively ductal location because of the extreme variation in anatomical and functional adaptation of these glands.

The secretion of kallikrein from duct cells into the saliva is increased by sympathetic nerve stimulation acting via α-adrenergic receptors in the cat (16, 144, 215), rat (186), and mouse (227).

The duct cells of the submandibular gland are also the storage sites of various trypsin-like esteropeptidases (36, 56), renin (44, 86), nerve growth factor (NGF) (222, 228), epidermal growth factor (EGF) (85, 259), lysozyme (125), and erythropoietin (59). The available evidence indicates that at least some of these substances, e.g., NGF and EGF, contrary to older views, are, like kallikrein (144, 216), secreted into the saliva (34, 173, 259) rather than into the blood circulation. NGF, like kallikrein, is also released by adrenergic stimulation (228, 262).

C. Pancreas

In 1965, Yasuda and Coons (285) confirmed the presumed localization of amylase, chymotrypsin, and trypsin in granules of the acinar cells of the pancreas of the pig by an immunofluorescence technique. Since then the same result has been obtained for kallikrein in the rat (16), pig (51), cat, and dog (153). Dorn et al. (55) recently have found immunocytochemical evidence of "trypsin-like activity (TLA)" not only in the acinar cells of the rat pancreas but also in the epithelial cells of the ducts and in the endocrine islet cells. Their electron microscopic studies showed immunoreactive granules in the β-cells of the islets of Langerhans. They suggest that these trypsin-like enzymes might have the specific proteolytic activity to process the conversion of proinsulin to insulin (234). The recent conclusion of Yoi et al. (285a) that human pancreatic kallikrein (like insulin) is confined exclusively to the beta cells is for the present difficult to reconcile with a variety of earlier observations discussed in Sections A and C above.

D. Kidney, Coagulating Gland, and Sperm

In the rat, kidney kallikrein has been localized to the cells of the distal tubules (188). Analysis of kidney homogenates indicates that it is bound to plasma membrane (263).

In the coagulating gland of the guinea pig, the kininogenase CGK was detected by immunochemical techniques to be concentrated luminally in cells lining the crypts (217). Its function and physiological substrate are unknown. Unlike acrosin in sperm, CGK appears to exist in the gland entirely in an active form (23, 168).

In sperm, acrosin has been located in the acrosomal region of the sperm head, possibly on the inner acrosomal membrane (171). A similar localization was concluded from studies on disruption and analysis of sperm cell fragments (76).

X. Kallikreins and Cell Proliferation

A. Kallikreins and Growth Factors

In the last few years evidence from various sources has suggested a possible role of kallikreins and other serine proteases in growth and developmental processes. Rixon, Whitfield et al. (192, 200) have suggested that kallikrein released from injured tissues may act as a local growth control agent or "wound hormone." This suggestion arose from their findings that the injection of pancreatic kallikrein into rats increased mitotic activity in cells of the thymus gland and bone marrow. Also Mandel et al. (140) reported that the systemic administration of kallikrein promoted wound healing and increased the survival rate of X-irradiated rats.

It is apparent from section IX of this review (SUBCELLULAR LOCALIZATION OF KALLIKREIN) that, in the submandibular gland, kallikrein and related esteroproteases are all localized in duct cells, possibly in the same secretory granules. In fact, there seem to be at least seven such related proteases in the mouse submandibular gland (27, 56, 220, 281) and at least as many different growth factors (see 27). In a concise but very pertinent publication and analysis in 1974, Pasquini et al. (191) pointed out that EGF probably existed in a complex with a binding protein, the latter an esteroprotease with hypotensive activity like kallikrein. From their studies on isolated secretory granules from the submandibular glands of mice, Pasquini et al. emphasize "that growth factors and esteroprotease activity are associated with the same intracellular granules, suggesting a possible role of the enzyme in the maturation of native molecules of growth factors as described for proinsulin-insulin conversion." From their more recent studies, Bothwell et al. (27) similarly conclude that "kallikrein, the γ subunit (of NGF), and EGF-binding protein may be only a few members of a large and closely related family of enzymes functioning in the proteolytic processing of biologically active polypeptides." Similar views have also been expressed by the reviewer (207, 208). The kallikreins may have various roles related to growth agents. For example, Lembach (136) has described the synergistic enhancement by the EGF-binding arginine esterase (a kininogenase) and mouse EGF on human fibroblasts. Similar results were obtained with thrombin instead of this arginine esterase (30). Possibly these enzymes activate the growth factors, or alternatively alter the cells so that they are more sensitive to growth factors.

B. Kallikreins and Growth, Differentiation, and Development

Plasminogen activators are serine proteases with specificities resembling trypsin (7). Interest in plasminogen activators and fibrinolysis has grown because of more accurate enzyme measurements and because of the wide distribution of these enzymes in cells and tissues. Plasmin itself has kininogenase activity (214).

Strickland (238) considers that plasminogen activation may play a role in physiological processes involving localized and regulated tissue breakdown that accompanies ovulation, embryo implantation, and aspects of early embryogenesis such as the invasion of uterine tissue by trophoblasts. Although the invasive period of the trophoblast is relatively short, about four or five days, Strickland points out that this is the period associated with the secretion of plasminogen activator by these cells. Obviously these observations extend the possible bioregulatory implications of fibrinolytic serine proteases far beyond their known role in haemostasis.

In a recent study, Denker and Fritz (50) point out the close chemical similarity between the rabbit blastocyst proteases and the glandular kallikreins.

C. Kallikreins and Neoplasia

Neoplastic cells grow to higher densities and are more agglutinable by plant lectins than are normal cells (see 83). Burger (31, 32) has shown that treatment with trypsin or bacterial serine proteases makes normal cells more agglutinable, apparently because their membranes become more similar to those of transformed cells. Burger (32) suggests that treatment with proteases temporarily releases certain normal cells from contact inhibition and induces a cell membrane surface characteristic of transformed cells.

In the neoplastic transformation of many types of cells there is a clear association between transformation on the one hand and increased production of plasminogen activators and the resulting fibrinolysis on the other (199). Danö et al. (46) have further shown that transformation of cultured fibroblasts by oncogenic viruses is associated with the release not only of plasminogen activators but of a range of other serine proteases.

Protease inhibitors have been reported to prevent neoplastic transformation in vitro (12) as well as to inhibit carcinogenesis in vivo (101, 257). More recently it has been reported that the protease inhibitors of microbial origin, antipain and leupeptin, not only suppressed radiation-induced cell transformation but also radiation transformation enhanced by 12-O-tetradecanoyl-phorbol-13-acetate (TPD), both in vitro. It was suggested that this action of protease inhibitors may be due to inhibition of an error-prone DNA repair process and may account for their reported suppressive effects on carcinogenesis in vivo (121).

XI. Kallikreins as Bioregulatory Serine Proteases

A. Homology of Kallikrein and Other Serine Proteases

In addition to the enzymes classified as kallikreins and kininogenases, many other serine proteases of diverse distribution and function are now known from chemical analyses to have extensive shared amino acid sequences. This includes the kallikreins, kininogenases, trypsin, chymotrypsin, elastases, thrombin, plasmin, plasminogen activators, $C\bar{1}$ protease, collagenases, coagulating gland kininogenase, acrosin, and others (176, 177, 207, 208, 239). There are also the structurally closely related proteases of bacterial and invertebrate origin. Of the latter, for example, cocoonase is a trypsin-like enzyme secreted in the cocoon by the silk gland of moths of the genus *Antherea.* At the appropriate time a solvent is secreted that permits the enzyme to hydrolyze the matrix of the cocoon, allowing the moth to escape (112, 113). Amino acid analysis indicates that bovine and porcine trypsinogens are at least as similar to cocoonase as they are to each other. Kafatos et al. (112) conclude that this supports the view of a common ancient ancestry for trypsin-like enzymes in insects and vertebrates.

There is also evidence of homology between certain bacterial serine proteases and those of invertebrates and vertebrates. Further, some of these bacterial serine proteases are kininogenases (196). *Streptomyces griseus* protease B and α-lytic proteases, *Sorangium,* have sequence homologies and relevant tertiary structure configurations that, when compared with mammalian pancreatic serine proteases, indicate that these enzymes, even those from bacteria, share a common ancestry with those of insects and mammals (8, 110, 111). Another interesting example of a family of serine proteases with trypsin-like actions and kininogenase activity among the prokaryotes are the subtilins, derived from *Bacillus subtilis* (196). In this instance, however, the family of subtilin enzymes differs from the trypsin family in having completely different amino acid sequences and three-dimensional structures, indicating unrelated evolutionary origins. However, the active sites of these otherwise different molecules have their catalytically functional groups in the same geometric relationship, perhaps an expression of the evolution of the same function at a molecular level (130, 229).

B. Diversity of Functions of Kallikreins

It is noteworthy that interesting speculations on the biological role of serine proteases have recently come from chemists. Stroud (239) and Neurath and Walsh (176, 177) have written with some eloquence on the molecular evolution of serine proteases and on their probable wide significance in biological regulation via limited proteolysis.

There is no doubt of the involvement of kallikreins and related enzymes in serial enzyme conversions and "cascade" processes such as occur in blood coagulation (figs. 2 and 3). New evidence indicates their likely involvement in fertilization of the ovum via acrosin, in ovulation, implantation, and possibly in cell growth, differentiation, and other developmental processes (see section X, KALLIKREINS AND CELL PROLIFERATION).

The serine proteases are known to activate proenzymes such as trypsinogen, chymotrypsinogen, etc., and plasma kallikrein itself activates pro-Hageman factor (figs. 2 and 3). It has been shown recently that urinary (or renal) kallikrein is a potent activator of plasma prorenin (225, 226). There is evidence that kallikrein-like proteases with their remarkably strict specificity regulate the conversion of prohormones like proinsulin and proglucagon in the pancreas (55, 234). Similar evidence exists for the conversion of proparathyroid to parathyroid hormone (88). Recently Straus et al. (236) have purified an enzyme from brain that is very similar to trypsin but distinguishable by its greater specificity. This enzyme cleaves "big" cholecystokinin CCK-33 at the Arg-Ile bond to yield CCK-12 and at the Arg-Asp bond to yield CCK-8. It may be that similar prohormone enzyme activators exist as a general phenomenon (236).

Other physiological or biochemical events possibly regulated by serine proteases via limited proteolysis are being reported. Not only is the activation of a number of procollagenases thought to be regulated by such a mechanism (282) but there is evidence that the biosynthesis of collagen itself is regulated by a series of limited proteolytic events (26). In skeletal muscle a highly specific protease, CAF (Ca^{++}-activated factor), may have a role in the metabolic turnover of myofibrils. CAF is capable of removing Z-disks from intact myofibrils. The enzyme degrades tropomyosin and troponin but has no apparent effect on myosin, actin, and α-actinin. There is evidence that CAF increases in atrophying muscle (123), which is taken to support the hypothesis that CAF is involved in myofibril turnover (47). In nerve, the modification of Na conductances by serine proteases has been demonstrated during perfusion of crayfish (233) or squid (205) axons. In transport systems, Moriwaki et al. have presented evidence that the pancreatic kallikreins enhance the intestinal absorption of amino acids (167) and glucose (166).

Although there is some uncertainty about the significance of the multiple molecular aggregates or forms in which NGF and EGF exist, there can be no doubt about their close association with kallikrein-like activity both chemically and in cellular localization (27, 137, 191, 217, 222, 227). The recent description of NGF in the prostate gland of the guinea pig (95) provides further evidence of the association of these two activites since the guinea-pig prostate, like the related coagulating gland, contains high concentrations of the kininogenase CGK (23, 168, 169, 217). This suggests a role, possibly interrelated, of these two agents in the reproductive tract. Orenstein et al. (185) have found that an active form of NGF is also a serine protease and that a feature of its restricted proteolytic activity is that it is a plasminogen activator. They describe this as the only known action of NGF on a nonneural substrate and speculate whether NGF in

mouse saliva may have a physiological role related to activation of plasminogen somewhere in the digestive tract. EGF, which is similar to NGF in localization and also in its association with kininogenase activity, has been the subject of new and interesting findings. Elder et al. (57) have shown that EGF is located in the ducts of the submandibular gland of man and appears to be closely similar, or identical to, urogastrone, which is located in Brunner's glands in the duodenum. (EGF and urogastrone react immunologically.) Elder et al. (57) ask whether EGF/urogastrone may play a physiological role in cell proliferation and wound healing in the gut. Other physiological possibilities, however, cannot be excluded. Like the names kallikrein or Kreislaufhormon, which have with time come to denote a group of serine proteases with diverse functions, NGF and EGF, originally regarded as specific growth hormones, also may have multiple and diverse functions.

The possible interrelationships of NGF, EGF, and kallikreins have been raised by Pasquini et al. (191), Shooter et al. (27), and by the reviewer (88, 144, 217). The earliest comment on the interesting association of these substances, however, was probably made by Levi-Montalcini et al. (137) in 1972: "The high concentration of kallikrein and kinins in snake venoms and in mouse salivary secretion as well, parallel to the high NGF concentration, again poses the question as to whether or not the two classes of biologically active substances are somehow related." The prostate gland of the guinea pig, long known to be a rich source of kallikrein (CGK) (13, 23, 169, 212, 217) has also been reported recently to be the same for NGF (95).

XII. Comments

In a symposium on kininogenases and kinins held in 1968 (241), the "Introduction" ended as follows: "It seems that the biological significance of the kinins and of the endogenous agents which release them is far from clear. Their major significance may well lie in a biological system which is not apparent to us." In the last decade, however, progress in the chemistry of the kallikreins has created a qualitative change in our knowledge so that we can now construct a conceptual biological framework into which the kallikreins fit comfortably, despite the fact that our knowledge of their biological and physiological roles is only beginning. The kallikreins must now be regarded as a group of enzymes belonging to the numerous and widespread serine proteases. The serine proteases, which are so similar in chemical structure and in their catalytic mechanisms that they are considered by some to have arisen from a common ancestral molecule, are, nonetheless, most diverse in their respective functions. Their relevant property is the proteolytic specificity whereby they exert their bioregulatory actions. The presence of their inhibitors in many mammalian cells and secretions and their wide distribution in nature reflect the ubiquitousness and probably the significance of the enzymes themselves.

Current evidence implicates serine proteases, including kallikreins, in conversions of proenzymes to enzymes, e.g., in the "cascade" of serial enzyme conversions in blood coagulation and in the homeostatic or self-regulatory digestive processes that convert trypsinogen, chymotrypsinogen, procarboxypeptidase, etc., to their active forms. Similarly, they are probably involved in the conversions of prohormones to the active hormones, e.g., of proinsulin, proglucagon, proparathormone, pro-NGF, and possibly of precursors of gastrin, cholecystokinin, and others.

The known properties of these enzymes further qualify them for functions in fertilization, in ovulation, and in implantation of the ovum; also, in cell growth and in embryonic development and differentiation. The possibilities are indeed extensive.

Future investigations probably will be concerned with local control processes in or between cells, involving cell growth, cell differentiation, locomotion, intercellular communication, etc. The development of dynamic and specific ultramicromethods of enzyme measurement will be essential.

Finally, the classification or nomenclature for kallikrein and related proteases must be adapted to advances in enzyme chemistry that increase our understanding of their catalytic mechanisms, their molecular evolution, and other properties. Reclassification or alterations in terminology may, therefore, be desirable in the future.

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