# KININS—A GROUP OF ACTIVE PEPTIDES[1]

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The term kinin refers to a number of peptides which have been classified as a group because they have a variety of potent pharmacological properties in common. All kinins are hypotensive, they contract most isolated smooth muscle preparations *but* relax the rat duodenum; they increase capillary permeability, produce pain when applied to a blister base on human skin, and cause bronchoconstriction in the guinea-pig which is specifically antagonized by salicylates and related compounds. Other peptides, such as oxytocin, vasopressin, substance P, angiotensin and eledoisin, share some properties with kinins, but all of these differ in, or lack, one or more of the properties listed above (1).

Progress in our knowledge of the pharmacological and chemical properties of kinins has been rapid in the last decade and these properties have been extensively described in a number of reviews (2 to 11). The reader is referred to these for detailed information. The present review, therefore, is primarily concerned with very recent information and with the more controversial aspects of these compounds.

## Discovery and Terminology of Kinins

The first evidence that a smooth muscle stimulating substance was released from an inactive precursor by an apparently enzymic action was obtained in 1937 when Werle et al. (12, 14) showed that the addition of kallikrein to serum resulted in the rapid release of a substance which contracted isolated smooth muscles. They called the latter compound substance Dk. to indicate its pharmacological property—*darmkontrahierende* Substanz. This demonstration preceded by two years the analogous one which showed that renin owed its hypertensive activity to its ability to release a peptide, hypertensin, from an inactive precursor in plasma (15, 16). Werle & Hambuechen (14) in 1943 drew attention to the similarity between the indirect pharmacological actions of renin and kallikrein, although in the former case the hypertensin released raised the blood pressure, whereas in the latter case the substance Dk. lowered it. In 1948, Werle & Berek (17) suggested that the name substance Dk. should be changed to kallidin and that its precursor (or substrate for kallikrein) should be called kallidinogen.

In 1949, Rocha e Silva, Beraldo & Rosenfeld (18) described the release of an active peptide from serum globulin by trypsin or snake venoms. They named the peptide bradykinin because it caused a relatively slow contraction of the isolated guinea-pig ileum. Although these workers distinguished this substance from acetylcholine, histamine, adenosine and other endoge-

[1] The survey of the literature pertaining to this review was concluded in July 1963.

nous active substances (including kallikrein), they were unaware that kallikrein had already been shown to release the smooth muscle stimulant, substance Dk. (or kallidin), from a precursor in plasma.

It is now clear that the kallikrein-kallidinogen-kallidin system and the typsin-bradykininogen-bradykinin system are very closely related (2 to 11). The reviewer does not share the opinion of Lewis (19) that Werle and his colleagues "did not appear to appreciate that kallikrein itself was an enzyme and acted by the formation of this substance. This realization did not come about until 1949. . . . " The work of Werle and his colleagues carried out from 1937–48 indicates clearly that they appreciated the fact that kallikrein acted enzymically to release kallidin, and that the latter substance accounted for many of the pharmacological properties of kallikrein.

A number of peptides closely related to kallidin and bradykinin were soon discovered. Several such compounds were found in wasp and hornet venom in a free and active form (20 to 23). These venom peptides were distinguished from kallidin and bradykinin, but because they resembled them so closely, Schachter & Thain (21) introduced the generic term ***kinin***, and the names wasp kinin and hornet kinin have been generally used for these peptides. Also, a similar peptide has been found in a free form in human urine and has been called urine kinin (24 to 25).

## Pharmacological Properties and Assay of Kinins

The main pharmacological actions of kinins have been listed in the first paragraph of this review and they are covered extensively in the reviews referred to in the introduction. The biological potency of kinins is, in general, comparable to that of acetylcholine, adrenaline, oxytocin, histamine, and other active endogenous agents.

The most satisfactory preparation for the assay of kinins is the isolated guinea-pig ileum which they contract. The assay is simple, sensitive, relatively constant in response to repeated doses, and the effects of acetylcholine and histamine are readily blocked by suitable antagonists. The isolated rat uterus is approximately 10 times more sensitive than the guinea-pig ileum and is useful when extra sensitivity is required. Its lesser specificity and poorer quantitative discrimination, however, offset this advantage. The isolated rat duodenum which relaxes to kinins in low concentrations may be useful in distinguishing them from other peptides such as substance P, which contracts this preparation, or from angiotensin, oxytocin and vasopressin, which are more than 1000 times less effective on a weight basis. The arterial blood pressure of all mammals so far tested is lowered by kinins (14, 22) and this test may be used in conjunction with others as an aid to identification. As a single test or assay system it lacks the simplicity, sensitivity and accuracy of the *in vitro* tests. Bronchial constriction occurs regularly in the guinea-pig after the intravenous injection of kinins. This effect is specifically antagonized by salicylates (26), a property which may be useful in differentiating kinins from other compounds.

## Structure, Release and Inactivation of Kinins

*Bradykinin (kallidin-9) and kallidin-10.*—The successful isolation of pure bradykinin obtained by the action of trypsin on acid-treated bovine serum globulin was first reported in 1960 by Elliott et al. (4). These workers concluded from their degradation studies that it was an octapeptide with the structure H.Arg-Pro-Pro-Gly-Phe-Ser-Phe-Arg.OH. Boissonnas and his colleagues then synthesized this octapeptide and a number of closely related analogues. They found that this compound was practically inactive biologically. However, one of their synthetic compounds which was identical with the octapeptide, but which contained an additional proline molecule in position 7, viz. H.Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg.OH, possessed potent bradykinin-like activity (11, 27). Elliott and his colleagues therefore reexamined their data and concluded that the natural bradykinin was, in fact, a nonapeptide with this structure (11, 28). The probable explanation for their original error is that the third molecule of proline was partially destroyed during hydrolysis of the pure peptide (11).

Shortly after the structure of bradykinin was elucidated, Pierce & Webster (29) isolated the peptide(s) released by the action of human *urinary* kallikrein on human plasma. They isolated and identified two peptides after this reaction, one kallidin-9 (identical with bradykinin), and another kallidin-10, which had the same structure but with an additional lysine molecule in the N-terminal position. Kallidin-10 is also released when salivary kallikrein acts on ox serum (30). In the latter instance 10 percent of the total kallidin-like activity was due to kallidin-9 which is thought by these authors to be formed, not by salivary kallikrein, but by the activation of enzymes in plasma during its acidification in preparing the substrate. The same authors showed that human *plasma* kallikrein, however, released only kallidin-9 from kallidinogen. Also, since plasma kallikrein failed to convert kallidin-10 to kallidin-9 they conclude that plasma kallikrein releases kallidin-9 directly from kallidinogen. Since plasma contains an aminopeptidase capable of converting kallidin-10 to kallidin-9 (31) it is apparent that kallidin-10 may be converted to kallidin-9 *in vivo* in an analogous way to the conversion of angiotensin I to II. However, since some of the biological actions of these peptides, e.g. hypotension, is more pronounced with kallidin-10 than with the nonapeptide, the decapeptide itself must be biologically active (31). The sites at which different enzymes act and convert the deca- to the nonapeptide, or inactivate these peptides, is shown in Figure 1.

The precursor of the different kinins appears to be the same molecule, at least for various kallikreins, trypsin and some snake venoms (32, 33, 34). It can be concentrated in fraction IV-6 of plasma by Cohn's fractionation method and behaves as an $\alpha$-globulin electrophoretically (32). Since the capacities of trypsin and kallikrein to attack synthetic esters and to release kinin are parallel, Elliott (33) has recently suggested that the release of kinin from the natural globulin substrate might occur by the splitting of an

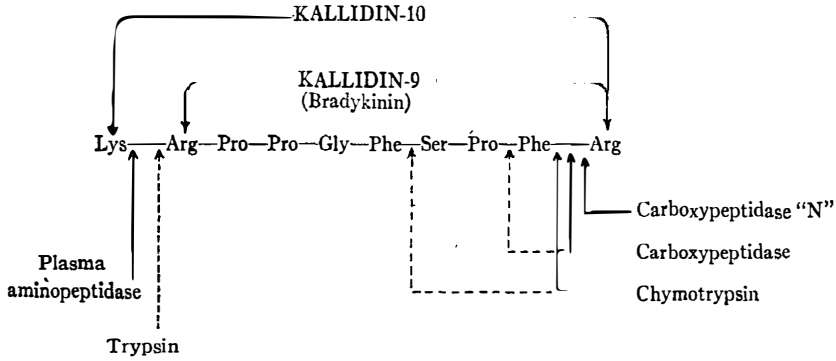


FIG. 1. Degradation of kinins by various enzymes (dotted lines indicate weaker action).

*ester* rather than a peptide bond. This could occur if the kinin moiety were involved in an ester bond either formed by the hydroxyl group of serine with the carboxyl group of another molecule or if the C-terminal carboxyl group was combined with the hydroxyl group of a polysaccharide. This interesting possibility seems unlikely for various reasons: (*a*) The inhibition of various kallikreins by some inhibitors is competitively prevented by benzoylarginine amide as well as by arginine esters (34). (*b*) Trypsin and thrombin which are known to split only peptide bonds in proteins, like the kallikreins, also have esterolytic activity on synthetic substrates (35). Werle & Trautschold (36) have demonstrated that agents such as DFP and methylene blue inactivate kallikrein and suggest, therefore, that histidine and serine form part of the "active site" required for the release of kallidin by kallikrein.

*Kinins produced by acid treatment of plasma.*—Kinins may also be produced in plasma by various physical and chemical procedures, possibly through the activation of kallikreinogen or other enzymes. For example, kinin-like activity appears in plasma by simple dilution (37), by contact with various foreign surfaces, and by changes in pH (38).

Various workers have found that if plasma is acidified to a pH value of 2–3.5, kinin is "spontaneously" released if it is then neutralized to pH 7–7.5 (without added enzyme) (9, 11, 33). These authors have isolated both kallidin-9 and kallidin-10 from acid-treated plasma, although the relative amounts of each peptide found by the different workers varied greatly. These differences may depend on different conditions of pH, or possibly on whether bovine or human plasma was employed. Recently, Elliott, Lewis & Smyth (39) have found a third kinin which develops in acid-treated plasma which appears to be kallidin-10 plus a methionine molecule added to the N-terminal lysine. This compound could be called kallidin-11 or methionyllysylbradykinin. Werle (11) suggests that kallikrein becomes active when plasma is acidified.

*Release from plasma by dilution and surface contact.*—The serum or plasma of many mammals (especially ox and guinea-pig) produces a kinin on dilution with Tyrode solution. Since this effect is prevented by soya bean trypsin inhibitor (which inactivates serum kallikrein), or by heating plasma for 3 hours at 56–60° (which destroys kallikreinogen), this release of kinin by dilution may be due to the activation of serum kallikreinogen which then releases kallidin from its substrate in plasma (4, 37).

Human plasma also generates a kinin on contact with glass and other foreign surfaces (40). Since this kinin caused pain when applied to a blister base on human skin it was called PPS (pain producing substance). Margolis (41, 42) found that kinin does not develop on contact with foreign surfaces with plasma of patients with "Hageman trait" (a condition characterized by a greatly prolonged clotting time in glass vessels but in which there is no abnormal tendency to bleeding). Margolis attributed kinin formation on contact of plasma with foreign surfaces to activation of a substance which he called component A (or contact factor) and he suggests that component A cannot be activated in this way in the absence of Hageman factor. Schachter and his colleagues (4, 43) have suggested that activated component A is closely related, if not identical, with serum kallikrein. More recently (11), Margolis has also adopted this view. Glass contact releases only a small portion of the kinin available for release since the different kallikreins, or trypsin, release as much kinin from plasma after, as before contact with glass (44).

*Kinin released by various extracts, secretions, etc.*—The release of kinin from an inactive precursor in plasma, or colostrum, is produced by a number of agents other than those described above. These are discussed briefly below.

(*a*) *Colostrokinin.*—A kinin releasing enzyme in the serum of man or ox becomes evident when serum is incubated with colostrum of women or cows (45, 46). In this case the enzyme is in the serum and the substrate in colostrum. The substrate, kininogen, increases in concentration in cow colostrum for about six days after calving and decreases slowly from the 10th day onwards. The significance of these findings is not known. The structure of colostrokinin is not yet established but it resembles the kinins of known structure in its pharmacological properties. Werle (32) suggests that the combination of colostrum and plasma somehow "releases" the enzyme activity in plasma.

Various kallikreins and trypsin also release a kinin from human and especially from bovine colostrum (32).

(*b*) *Ornithokinin.*—The pancreas and some other organs of birds (hen, duck), like those of mammals, contain kallikrein. This enzyme ornithokallikrein releases kinin from serum of birds, but not from serum of mammals. Conversely, kallikrein of mammalian origin does not release kinin from avian plasma (2, 30). The chemical structure of ornithokinin has not yet been determined.

(*c*) *Kinin released by bacterial enzyme.*—Prado et al. (47) found that "clostripaine," a cysteine-activated protease secreted by *Clostridium histolyticum*, released a kinin from horse serum which was indistinguishable from bradykinin in its pharmacological properties. They conclude (48) that this release is a direct action of "clostripaine" on serum kininogen and not mediated by the activation of plasminogen as suggested by Lewis (6). This kinin has not yet been purified.

(*d*) *Kinin released by sweat.*—Fox & Hilton (49) have shown that human sweat releases a kinin from dog pseudoglobulin, and also that kinin appears in a subcutaneous perfusate if the body temperature is raised by heating the trunk and legs. These authors conclude, therefore, that a kinin is formed in the interstitial fluid by the action of this enzyme in sweat, and that this mechanism fully accounts for the vasodilatation which occurs during sweating. In the reviewer's opinion this conclusion cannot yet be accepted unreservedly. It would be of interest to know whether subjects with congenital absence of sweat glands "release" kinin into a subcutaneous perfusate under the conditions described by Fox and Hilton. If they do, the presence of kinin in the perfusate must be independent of sweating.

(*e*) *Neurokinin.*—In a series of papers by Chapman et al. (50, 51, 52), it has been claimed that a kinin releasing agent and a kinin (neurokinin) appear in the CSF of patients during migraine and other neurological disturbances. The CSF of normal dogs and monkeys, however, contains neither kinin, kininogen, nor kinin releasing enzymes (44). Chapman and his colleagues also present evidence that kinin is released from cutaneous nerves after antidromic stimulation in man. These remarkable observations should be repeated and precautions taken to ascertain whether increased concentrations of kinin in perfusates or CSF under these conditions is perhaps secondary to vasodilatation, alterations in vascular permeability, or "spontaneous" formation of kinins by dilution or by contact of the test fluid with foreign surfaces.

(*f*) *Kinin release by an enzyme in accessory sex glands of the guinea-pig.*—A potent kinin releasing enzyme has recently been found in the coagulating and prostate glands of the guinea-pig (53). This enzyme releases kinin from plasma or serum of many mammals but not from that of the rat. The prostate glands of man, dog, cat, rabbit and rat, unlike that of the guinea-pig, fail to release kinin from their own or other plasmas. This is not in accord with the generalization that this enzyme regulates functional hyperaemia in all glands. More likely, in this case it has a function in the reproductive tract which is, perhaps, unrelated to its ability to release kinin.

(*g*) *Kinin release by "permeability globulin."*—The pharmacological properties of a globulin obtained by ether fractionation of guinea-pig plasma were described by Mackay et al. in 1953 (54). This fraction, also called G-2$\alpha$, increased vascular permeability and produced hypotension in various mammals (55, 56, 57). The close similarity and probable identity of this substance to serum kallikrein has been emphasized by Schachter and

his colleagues (4, 5, 43). An adequate direct comparison of the serum kallikreins and "permeability globulin" has unfortunately, however, never been made. Recently, Mason & Miles (56) have stated that the pharmacological effects of "permeability globulin" are probably due to release of kinin but that this globulin differs from serum kallikrein in that unlike the latter, it does not act directly on kininogen, but releases kinin by first activating a "kininogenase." These authors unfortunately compared the ability of *guinea-pig* permeability globulin and that of *human* serum kallikrein to release kinin from *ox* serum globulin. In the opinion of the reviewer, the apparent distinction of permeability globulin from the serum kallikreins by this criterion is not fully warranted, because killikreins from different species vary in their substrate specificities (58, 59). A comparison should be made, therefore, of preparations of these substances obtained from the same animal species.

The possible interrelationship and identity of kallikrein and other "factors" in serum is illustrated in Figure 2.

## Kinins Occurring in an Active Form

The kinins discussed above exist normally as a structural part of an inactive precursor and are probably released by the splitting of a peptide bond. A number of kinins do exist in an active or free form, however, in various venoms and also in mammalian urine. None of these has as yet been purified, but since their pharmacological properties are so similar to the known kinins they must be closely related chemically.

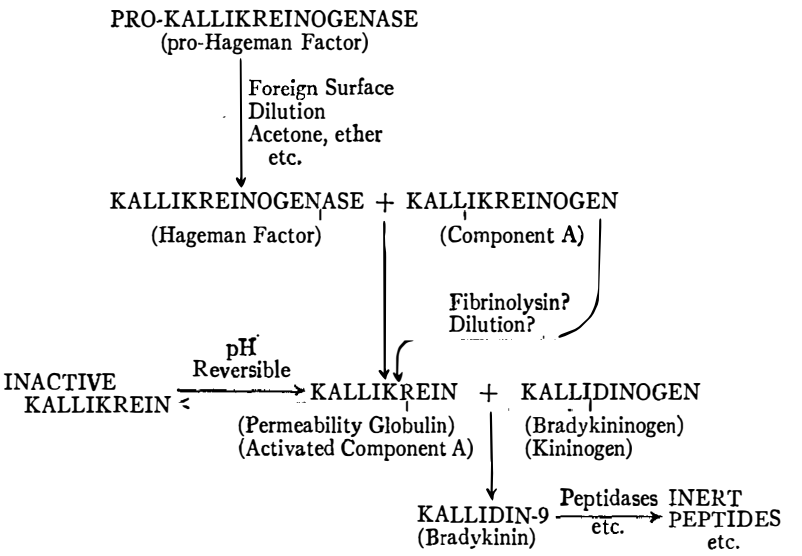


Fig. 2. Possible relationship of kallikrein and other factors in plasma.

*Urine kinin.*—A kinin was first described in human urine by Werle & Erdös (24). Because they thought that it differed slightly from their impure kallidin preparation they called it substance Z. This substance in urine was also described independently by Gomes (60) who concluded that it might be identical with bradykinin. Gaddum & Horton (25) renamed it urine kinin because they also could not distinguish it from other kinins. The amount of urine kinin excreted does not vary with changes in the pH of urine, sweating or prolonged salivation (61). The source of urine kinin and its significance are unknown.

*Venom kinins.*—The term kinin was first introduced for a substance in wasp venom because although it closely resembled bradykinin (kallidin-9) it differed from it in its inactivation by trypsin and in its chromatographic behaviour (20, 21, 22). Wasp venom may also contain two other kinins in low concentrations (62). The pharmacological potency of the impure major kinin suggests that it is more potent than the kallidins. Wasp venom also contains high concentrations of histamine and 5-hydroxytryptamine.

Like wasp venom, hornet venom (*V. crabro*) contains histamine (3–30 mg/g dry venom sac) and 5-hydroxytryptamine (7–19 mg/g). It differs, however, in that it also contains high concentrations of acetylcholine (10–15 mg/g) and a different kinin.

Since minor variations in the structure of bradykinin result in the loss of almost all biological activity (27) it would be of interest to know the structure of the venom kinins, particularly since wasp kinin may be more potent than those of known structure.

## Kinin Releasing Enzymes (Kininogenases) in Plasma

The findings that kinin-like activity develop in plasma on contact with foreign surfaces and on dilution, as discussed earlier in this review, raised the question which chemical system leads to kinin release under these and other conditions. In 1950, Beraldo (63) had found that crude preparations of bovine fibrinolysin (plasmin) released kinin from plasma. Later, Schachter (37) compared the kinin releasing action of fibrinolysin and various kallikreins and suggested that "the dilution of serum might simply dissociate the kallikrein-inactivator complex in plasma, or that it might activate serum proteases (like fibrinolysin) which in turn might release kallikrein." In further studies with highly purified preparations of human fibrinolysin, various kallikreins, and trypsin, Bhoola et al. (43) found no correlation, however, between their fibrinolytic activity and their ability to release kinin. The possibility that fibinolysin may indirectly lead to the release of kinin under certain conditions is not excluded, however. In fact, Vogt (64) has recently found that human fibrinolysin releases a kinin from dog serum globulin *in vitro*, not by acting on kininogen, but indirectly, by activating kallikreinogen. In the reviewer's experience, however, serum kallikrein is far more effective than fibrinolysin in releasing kinin. The systems involved in kinin

release and inactivation in plasma obviously rival those involved in blood clotting in complexity.

## Possible Physiological and Pathological Roles of Kinins

The properties of kinins and of the enzymes which release them are such that they could participate in blood flow regulation, inflammatory reactions, allergic phenomena, etc. Some of these possibilities have been discussed earlier, but some of particular interest are discussed below.

Hilton & Lewis (65 to 69) hold the view that kinin releasing enzymes pass from secretory cells into the interstitial fluid during activity, and thus regulate functional hyperaemia in glandular organs. These authors also conclude that true vasodilator nerve fibres to blood vessels do not exist in the salivary glands or tongue.

The view that kinin releasing enzymes provide a mechanism regulating functional hyperaemia in glands *generally* has been questioned by Bhoola et al. (53) who found that of a large number of glands examined in the guinea-pig, only the submaxillary and prostate glands contained a kinin releasing enzyme in an active form. Further, whereas this enzyme in the guinea-pig's salivary gland releases kallidin from the plasma of many mammals it fails to do so from guinea-pig plasma (70). Also, unlike the guinea-pig, a number of other mammals examined did not contain a kinin releasing enzyme in their prostate glands (53). In fact, the question also arises as to whether the function of these enzymes is necessarily related to their ability to release kinin. In this connection it is worth noting that it has never been suggested that the physiological function of trypsin is to release kinin, although it does this effectively. Salivary and pancreatic kallikrein may be exocrine secretions with functions analogous to that of trypsin.

Schachter and his colleagues (71, 72) have also re-examined the problem of whether kallikrein is the mediator of nerve-induced vasodilatation in the submaxillary gland. In their opinion the validity of this view, even in this instance, is open to question for the following reasons:

*In the cat:*—(*a*) It is possible to produce a maximal vasodilatation by stimulation of the chorda tympani nerve when the gland has been perfused for several hours with horse serum, from which cat saliva fails to release kinin. Under these conditions, the close arterial injection of dialysed saliva also fails to cause vasodilatation. (*b*) The close arterial injection of saliva into a gland with natural circulation cannot duplicate the vasodilatation produced by stimulation of the chordo-lingual nerve. The increased flow produced by the close arterial injection of saliva is always slower in outset, it is never as great as can be achieved by nerve stimulation (or by injected acetylcholine), and it is more protracted.

*In the rabbit:*—(*a*) Contrary to the case in the cat and dog, both the secretion and vasodilatation produced by stimulation of the chordo-lingual nerve are equally sensitive to inhibition by atropine, and they also recover in parallel. (*b*) Although atropine usually blocks vasodilatation produced by

chordo-lingual stimulation, the delayed vasodilatation after sympathetic nerve stimulation is unaffected. The parasympathetic and sympathetic vasodilatation, therefore, are not likely to be caused by the same chemical mediator.

Although the identity of the chemical mediator of nerve-induced vasodilatation is not established by the above experiments (71, 72), the results obtained are not consistent with the view that kallikrein mediates all functional vasodilatation in the salivary glands. They are consistent with the view that parasympathetic cholinergic vasodilator nerve fibres exist, however, if one assumes that the relative resistance of chordo-lingual induced vasodilatation in the submaxillary gland of the cat and dog to atropine is simply an extreme case of the well known variation in the sensitivity of cholinergic receptors to atropine. The rejection of the view that true vasodilator nerve fibres exist in the salivary glands and tongue is, in the reviewer's opinion, premature. This interesting problem requires further clarification. As with many other functions speculatively attributed to kinins, the discovery of a specific kinin antagonist would be a most valuable analytic tool in providing a more definite assessment of its role in functional vasodilatation.

Because of their availability for release following various types of tissue damage, the possibility that kinins contribute to certain aspects of some inflammatory, allergic, or anaphylactic phenomena is a definite possibility. Their exact contribution to specific types of "injury" reactions, however, has not been clearly established as yet.

There is evidence that hereditary human angioneurotic oedema is due to a deficiency of serum kallikrein inhibitor analogous to the absence of anti-haemophilic factor in haemophilia (73). There also appears to be some interrelationship between the kallikrein-kallidinogen-kallidin system of plasma and "Hageman trait" in man since "Hageman plasma" does not generate kinin on contact with glass (41, 42). These individuals, therefore, may be lacking some factor (kallikreinogenase?) which is essential to release kallikrein from its inactivator. These patients show no haemorrhagic disturbance but their blood has a prolonged clotting time *in vitro* (74).

The kinins in wasp and hornet venom must contribute to the inflammatory reaction following stings by these insects. The co-existence in some venoms of acetylcholine, histamine and 5-hydroxytryptamine with a kinin is of interest from a comparative biological view.

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