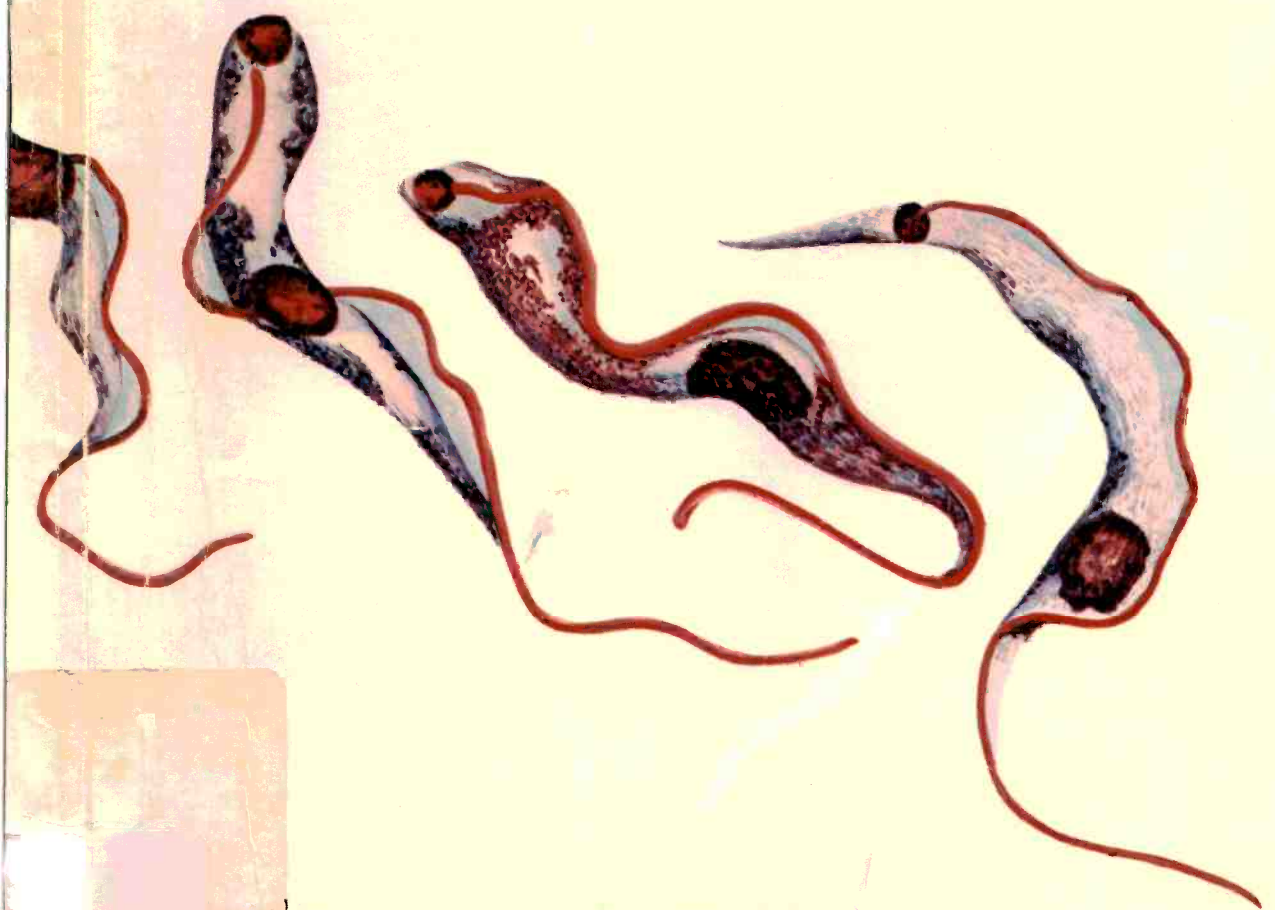
IDRC-132e

Pathogenicity of Trypanosomes

Proceedings of a workshop held at Nairobi, Kenya, 20-23 November 1978



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Editors: **George Losos[1] and Amy Chouinard[2]**

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Pharmacologically active substances in *T. brucei* infections

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Abstract. Pharmacologically active substances appear to be important in the pathogenesis of African trypanosomiasis. Urinary kallikrein increases in rabbits infected with *T. brucei* 3–4 days after infection but the mechanism is unknown. Plasma kallikrein is also activated with peak levels occurring 10–14 days after infection. Levels of circulating IgG immune complexes parallel the release of kallikrein, and it has been demonstrated in vivo and in vitro that immune complexes activate the kallikrein-kinin system probably by the absorption and activation of Hageman factor. Activation of Hageman factor causes not only the release of kinin mediators of inflammation but also activation of the coagulation, fibrinolytic, and complement systems that contribute to the pathogenesis of the disease. Increases in blood viscosity contribute to the hemodynamic changes seen in trypanosomiasis.

Human trypanosomiasis and the experimental disease caused by *T. brucei* are basically inflammatory conditions. Rabbits infected with *T. brucei* suffer inflammation after about 2 weeks, with their extremities such as ears, eyelids, external nares, and scrotum, being particularly affected. It is not surprising, therefore, to find that the pathogenic mechanisms are very similar to the inflammatory response and that the mediators of inflammation are involved in this disease.

During chemotherapeutic studies, Goodwin noted that nontoxic doses of drugs cured the parasitemia of some mice infected with trypanosomes but that often the animals died within 24 h or so (Goodwin and Richards 1960). This phenomenon reminded him of an earlier study by Stephan and Esquibel (1929) who had similar results when using acriflavine to treat cattle with piroplasmosis. Goodwin set out to investigate this phenomenon in *T. brucei* infections, trying to explain the pathogenic mechanisms of trypanosomiasis to suggest better methods of treatment.

Release of Kinins and Kallikrein

The earliest studies demonstrated increases in histamine and short-chain peptides related to bradykinin in the urine, blood, and tissues of infected mice (Goodwin and Richards 1960; Richards 1965). Similar results were also obtained in patients with severe burn trauma (Goodwin et al. 1963), suggesting that tissue damage was important. The release of pharmacologically active substances was not confined to trypanosomiasis, and similar results were obtained with mice infected with other protozoal infections, bacteria, and viruses (Goodwin and Richards 1960). At about the same time as these studies were undertaken, the kallikrein-kinin system was being extensively studied, and many new facets about the biochemical pathways and mechanisms of activation were being discovered. More importantly, the kallikrein-kinin system was being implicated in various disease processes, especially anaphylactic shock (Brocklehurst and Lahiri 1962), inflammation (Elliott, Horton, and Lewis 1960), rheumatic disease (Eisen 1970), and bacterial infections (Amundsen and Rugstad 1965).

Kinins were found to be released in rabbits and cattle infected with *T. brucei* and humans infected with *T. rhodesiense* (Boreham 1968a, 1970). Levels of the $\alpha 2$ globulin precursor kininogen decreased at the same time as kinin was detected in the blood. Similar studies in malaria and babesiosis showed that the kallikrein-kinin system was involved in these diseases as well (Onabanjo and Maegraith 1970; Wright 1973). In chronic trypanosomiasis infections, the release of kinins occurred 1–2 days after the first antigenic variant

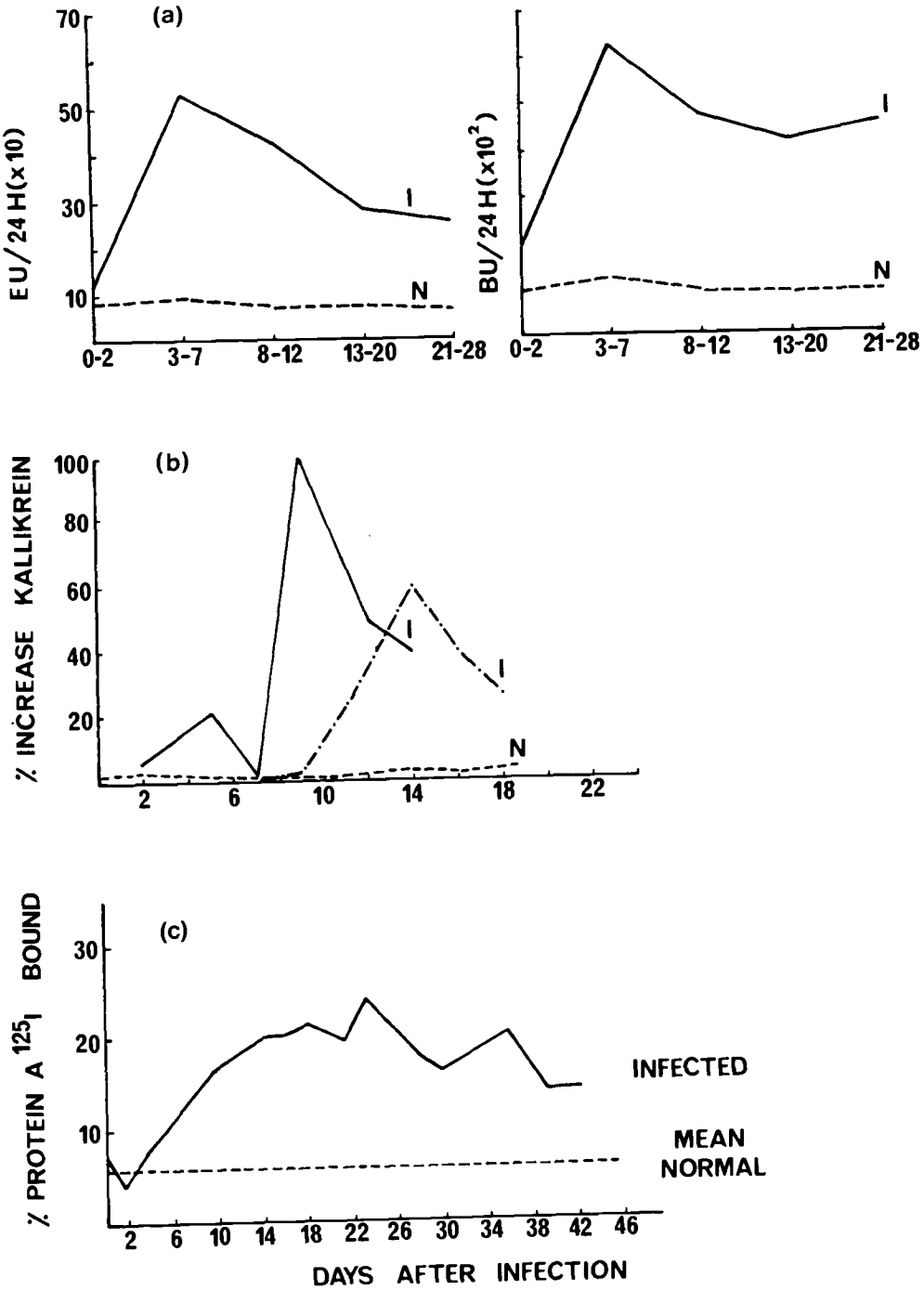

Fig. 1. Changes in urinary kallikrein (a), plasma kallikrein (b), and circulating IgG immune complexes (c) in rabbits infected with T. brucei.

of trypanosomes was produced, suggesting that there was an association between the two events (Boreham 1968a).

Kallikreins are enzymes normally present in inactive forms and found in the glandular organs, blood, lymph, and urine. Although similar in properties, the enzymes are different from one another. They have direct biological activities, such as chemotaxis, and they act on kininogen to form other biologically active substances — kinins. Plasma kallikrein cleaves high molecular-weight kininogen to form bradykinin, whereas glandular kallikrein forms kallidin (lysyl-bradykinin).

Samples of urine from rabbits infected with *T. brucei* have been analyzed for kallikrein activity at intervals during the infection (Fig. 1a); the results indicate that within 3–4 days of infection, urinary kallikrein activity increases and peaks at 4–8 times normal values after 6–10 days. Despite subsequent fluctuations, concentrations remain high throughout the infection (Wright and Boreham 1977).

Plasma kallikrein concentrations have also been measured, but they show a different pattern of results (Fig. 1b). Increases in plasma kallikrein occur after rises in urinary kallikrein and reach a peak 10–16 days after infection. Although the length of time varies, maximum plasma kallikrein concentrations are always detected after the urinary kallikreins are raised. This indicates that increases in urinary kallikrein do not occur as the result of increased filtration of plasma kallikrein through the kidney glomerulus. This is confirmed by studying the properties of urinary and plasma kallikreins released in trypanosome infections. Plasma kallikrein but not urinary kallikrein is inhibited by soybean–trypsin-inhibitor, whereas both plasma and urinary kallikreins are inhibited by the protease inhibitor aprotinin (Trasylol). Plasma prekallikrein levels show an inverse relationship to plasma kallikrein.

Mechanism of Kallikrein Activation

The evidence that immune complexes activate plasma kallikrein has recently been reviewed (Boreham and Wright 1976b). The most important pieces of evidence are that complexes cause the release of kinins in vitro from a kininogen substrate and that injection of immune complexes in vivo into rabbits causes a profound hypotension, which is inhibited by aprotinin. Trypanosomal antigen injected intravenously into rabbits with preformed antibody due to an infection also results in hypotension caused by the activation of the kallikrein-kinin system (Boreham and Wright 1976a,b). It seems probable that immune complexes absorb and activate Hageman factor (factor XII), which in turn activates prekallikrein. This is supported by in vitro evidence that heating the kininogen substrate to 65 °C but not 56 °C prevents kinin release by immune complexes. (Hageman factor is destroyed at 65 °C but not 56 °C.)

The author measured the concentration of circulating immune complexes in infected rabbits; he used staphylococcal protein A, which binds to the Fc portion of the immunoglobulin G molecule, as the basis of a radioimmunoassay for soluble IgG complexes (Crawford and Lane 1977). He found that the amount of circulating immune complexes increases significantly during infection (Fig. 1c), and the increase corresponds to the activation of kallikrein. Preliminary studies using SDS gel electrophoresis suggest that the antigen component of the immune complexes is nontrypanosomal in origin. This finding is, perhaps, not surprising because only about 5% of the IgG antibody produced in trypanosomiasis is trypanosome-specific (Freeman et al. 1970) and several auto- and heterophile antibodies are also present (Boreham and Mackenzie 1974). It is now well established that in *Babesia* infections of cattle, an esterase enzyme present in the parasite is responsible for kallikrein activation (Wright 1975). This enzyme, which will also convert fibrinogen to fibrin, probably acts directly on prekallikrein and not through an intermediate substance.

Although kallikrein is also activated during malaria infections, no attempts have been made to determine the mechanism. Because malaria is an intracellular parasite, one might expect a mechanism similar to *Babesia* infections. In vivo and in vitro experiments have demonstrated that a similar esterase enzyme does not occur in *T. brucei*.

Because urinary kallikrein concentrations are raised within about 3 days of infection, it seems unlikely that immune complexes are involved in its activation. Urinary kallikrein is known to be produced in the kidney, probably in the juxtaglomerular complex (Nustad 1970). Various mechanisms may be hypothesized to explain its release, such as circulating endogenous chemicals, increased kidney blood flow (possibly caused by plasma kinins released locally), or alterations in aldosterone concentrations (Keiser et al. 1976).

Kinins normally have a very short half-life (less than one complete circulation of the blood) due to the presence of kininase enzymes (Ferreira and Vane 1967), but they exist much longer when kininases are inhibited by slightly acid pH (Edery and Lewis 1962). In trypanosomiasis, the inflam-

matory exudates, which are acidic, may be responsible for local concentrations of free kinins.

No work on other pharmacologically active substances in *T. brucei* infections has been published since the review of Boreham and Wright (1976a). Because trypanosomiasis produces a great deal of stress to the host, one would expect catecholamine to be released (Goodwin 1976); however no depletion of epinephrine from the adrenal glands of rats was found during *T. brucei* infections. Only Yates (1971) has reported some depletion of norepinephrine from the hearts of rabbits chronically infected. The catecholamine metabolites do increase, but the significance of these changes is not known at present. It appears that changes in the catecholamines are certainly not as important in *T. brucei* as they are in *T. cruzi* infections. It has recently been shown that *T. cruzi*-infected rats experienced complete depletion of norepinephrine in the heart during the acute phase of infection and recovered during the chronic phase (Machado, Machado, and Gomes 1975; Machado, Machado, and Chairi 1978). This finding implies complete functional denervation of the adrenergic nervous system in acute Chagas' disease.

The importance of histamine and 5-hydroxytryptamine (5HT) as pharmacologic mediators varies with host species and tissue. The rabbit is very insensitive to histamine, whereas the guinea pig responds well to both agonists. There is no evidence to suggest that these substances are important in human or cattle disease caused by *T. brucei* subgroup organisms. At present, the kallikrein and kinins seem to be the most important pharmacologically active substances released. Almeida et al. (1977) showed that the hypotensive undecapeptide substance P is released in *T. cruzi* infections, but so far no similar studies have been undertaken in *T. brucei* infections. The roles of prostaglandins, angiotensin, and SRS-A have not yet been evaluated, although SRS-A has been implicated in acute systemic anaphylaxis in cattle (Eyre, Lewis, and Wells 1973) and may be an important mediator of pathogenic reactions in cattle trypanosomiasis.

Possible Effects of Kallikrein Activation

Kallikrein is activated early in *T. brucei* infection in rabbits, cattle, and humans and likely initiates a number of pathogenic processes. The kallikrein-kinin system is interrelated with the coagulation, fibrinolytic, and complement systems (Fig. 2). Activation of Hageman factor initiates reactions in all four systems. Thus, it is possible that the activation of Hageman factor by immune complexes accounts for the majority of the pathological symptoms. Many of the biochemical pathways activated are likely to produce secondary pathological changes in tissues and organs.

The biological effects of the kallikrein-kinin system are hypotension; increased capillary permeability; pain; chemotaxis of leukocytes; contraction of many isolated smooth-muscle preparations; and the release of catecholamines, histamine, and prostaglandins.

Severe hypotension is characteristic of rabbits infected with *T. brucei* and occurs early in infection (Boreham and Wright 1976a). Hypotension is not a common finding in human trypanosomiasis, although reports do exist (Sicé 1937; Buyst 1975). The most likely explanation of this anomaly is that the maintenance of blood pressure is a complex mechanism consisting of hormonal and neural components in which a balance is normally maintained. Thus, compensatory mechanisms exist to counteract the effects of kallikrein.

Increased capillary permeability is a consistent feature of trypanosomiasis. Kinins, like histamine and 5HT, bear a net positive charge on their molecules and probably combine with acidic groups on mucopolysaccharides and glycoprotein components of blood vessel walls, initiating changes in permeability. The consequences of altered permeability are great. Not only are plasma and lymph constituents allowed to escape from vessels but the quality of the fluid is also changed. For example, it has been shown that in inflammatory conditions the amount of protein in lymph increases from 1 to 8%. Early in the inflammatory reaction, tissue degeneration occurs, causing the release of metabolic substances that are mainly acidic. These substances raise the osmotic pressure outside blood vessels so much that the fluid within is withdrawn to dilute them, and edema results. Degeneration of tissues in trypanosomiasis has been demonstrated by Goodwin (1970, 1971).

One of the consequences of inflammation is the production of the acute phase beta globulin proteins known as C-reactive protein (CRP). Concentrations of CRP indicate the severity of the inflammation and the amount of damage caused. In trypanosomiasis, CRP levels have been shown to be raised in rabbits infected with either *T. congolense* or *T. brucei* (Thomasson et al. 1973; Cook 1979). The anti-inflammatory drug indomethacin prevents the increase in CRP levels and delays the onset of pathological symptoms caused by edema.

Plasma albumin decreases in trypanosomiasis. This may be the result of increased plasma volume (Boreham 1967; Clarkson 1968; Naylor 1971), or it

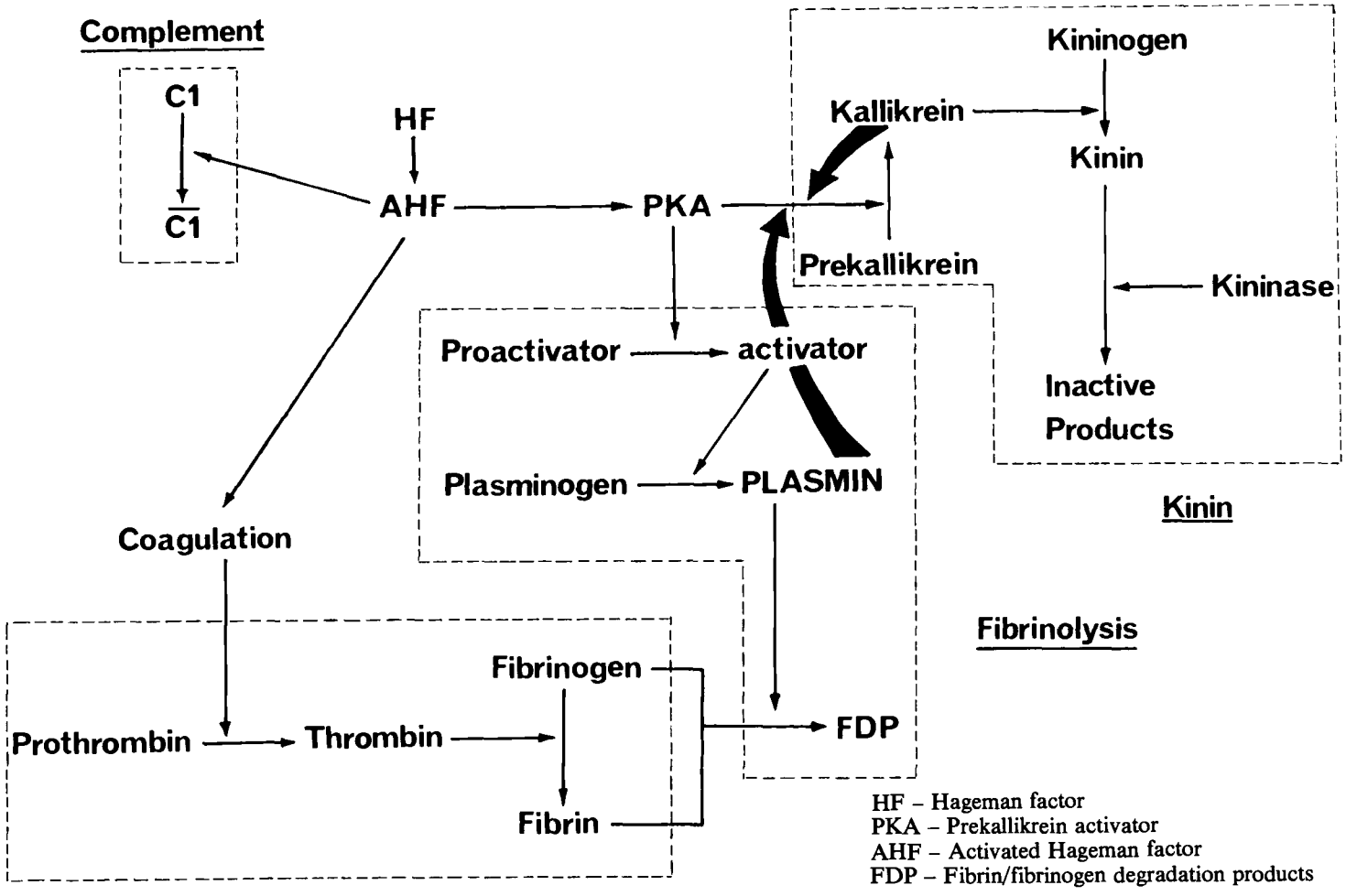

Fig. 2. Interrelationship of the kallikrein-kinin system with the coagulation, fibrinolytic, and complement systems. The solid arrows indicate feedback mechanisms.

may be due to depressed hepatic synthesis of albumin in inflammation (Page 1972). Because albumin is the major factor controlling the colloid osmotic pressure within blood vessels, its decrease would encourage edema.

Changes in coagulation have been noted during trypanosomiasis, although the effects are probably not very significant. Thrombocytopenia occurs (Davis et al. 1974; Robins-Browne, Schneider, and Metz 1975), and increases in some of the clotting factors, especially factors VIII and XII, have been reported (Boulton, Jenkins, and Lloyd 1974). Robins-Browne and Schneider (1977) reported that four out of five patients suffered from bleeding disorders early in trypanosomiasis infections and, interestingly, that suramin sodium initially aggravated the coagulation defects. The latter finding may be attributable to the drug's inhibiting kallikrein and complement activation and preventing the action of thrombin on fibrinogen (Eisen and Loveday 1973).

The fibrinolytic system is also activated in trypanosomiasis; at the same time, plasma fibrinogen increases significantly, contributing to the hyperviscosity syndrome seen in infected rabbits (Facer 1976). Fibrinolysis occurs in rabbits, demonstrated by the formation of breakdown products (FDP) of fibrin and fibrinogen as a result of plasminogen activation (Boreham and Facer 1974). The presence of FDP has also been reported in human trypanosomiasis (Greenwood and Whittle 1976a).

Several authors have shown decreases in C3 levels, indicating activation of complement in trypanosomiasis (Nagle et al. 1974; Greenwood and Whittle 1976b). Such activation results in the formation of chemotactic fragments C3a, C5a, and the C5b67 complex as well as the anaphylatoxic mast cell degranulating fragments C3a and C5a. One of the most important components produced when complement is activated is C3b, which enhances phagocytosis.

Chemotaxis of leukocytes is an important process in inflammation. It is known that the conversion of prekallikrein to kallikrein results in the formation of chemo-attractants for both neutrophils and human blood monocytes (Gallin and Kaplan 1974). Recent studies (Cook 1977) have indicated that in vitro immune complexes of trypanosomes and antibody, but not trypanosomes alone, generate chemotactic activity that is not complement-dependent. Trypanosomes and immune sera sensitize isolated tissues to agonists such as bradykinin, acetylcholine, and histamine (Boreham and Wright 1976b). One possible explanation of this observation is that trypanosomes or their products cause a local release of prostaglandins.

The major event in the pathogenesis of trypanosomiasis caused by *T. brucei* subgroup organisms is the activation of Hageman factor by immune complexes. This occurs approximately 10 days after infection when sufficient antigen and antibody are present. Activated Hageman factor initiates chain reactions that produce the signs and symptoms of trypanosomiasis, the most important of which seems to be the alteration in capillary permeability. The hyperviscosity syndrome that occurs as a result of changes in red-cell structure and increased plasma globulins and fibrinogen causes sludging of the blood in the microvessels and further kinin activation with the formation of microthrombi. When immune complexes are produced, they have direct pathological effects as they are deposited in the glomerulus (Houba 1977). The pathogenesis of trypanosomiasis is not unique but is similar in many respects to other inflammatory diseases such as rheumatoid arthritis and glomerulonephritis.

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