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Biologically Active Products from African Trypanosomes

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INTRODUCTION

In spite of the rapid development of medicine in recent years, the pathogenic trypanosomes continue to maintain their lethal grip on both humans and domestic animals over much of tropical Africa. There are a number of reasons for this. First, it is only relatively recently that the diseases caused by these organisms have been intensively studied with relatively sophisticated techniques. With this has come the realization that the diseases caused by different trypanosomes, although they have certain features in common, tend, in general, to be highly variable and possibly differ widely in their pathogeneses. The precise form of each disease depends not only upon the host animal species and on the species of infecting trypanosome but also upon the particular strain of trypanosome involved (100). Second, trypanosomes fail to produce, in either humans or domestic animals, gross lesions which could be considered to be obviously lethal. As a result, it is not known why animals die. One explanation for this situation is that the diseases may be produced by toxins. However, although the existence of trypanosome toxins has been hypothesized for very many years, it was as recently as 1974 that an eminent researcher in this field claimed, with some assurance, that "there is no evidence that trypanosomes produce toxins of any kind whatsoever" (53). The situation has changed in recent years, and it has been demonstrated that trypanosomes may release a number of biologically active factors. The importance of these factors is not firmly established, yet, when acting together, they may account for the lesions observed, for the various clinical features of these diseases, and for the deaths of infected individuals.

African Trypanosomes

Five "species" of trypanosome are of major importance in central Africa. Two of these, *Trypanosoma brucei gambiense* and *T. brucei rhodesiense*, cause disease in humans. They are morphologically indistinguishable, but *T. brucei gambiense* causes a chronic, slowly progressive neurological disease known as sleeping sickness, whereas *T. brucei rhodesiense* causes an acute, rapidly lethal, febrile disease in which neurological signs may be relatively insignificant (4).

The principal trypanosomes which cause disease in domestic animals in sub-Saharan Africa are *T. congolense*, the most important cause of bovine trypanosomiasis in East Africa, and *T. vivax*, which is considered to be of greater importance in West Africa (100). Nevertheless, *T. vivax* has caused serious losses in East and central Africa in the past. The third important trypanosome to infect cattle is *T. brucei brucei*. This organism is somewhat less pathogenic for domestic animals than is either of the other two species. These three species are widespread throughout tropical Africa, and it is not unusual for cattle to be infected with two or three species of trypanosome simultaneously.

The trypanosomes of camels (*T. evansi*) and of horses (*T. equiperdum*) will not be considered in this review. Although they are of considerable economic significance in North Africa, they do not require the tsetse fly for transmission and are biologically quite distinct from the trypanosomes described above.

Trypanosomiasis

Clinical signs. Gambian sleeping sickness in humans is characterized by a relatively long incubation period followed by the gradual but progressive onset of irregular fever; anemia; splenic, hepatic, and lymph node enlargement; and cachexia (4, 138). Affected individuals eventually show neurological disturbances, including headaches, hyperesthesia, changes in mental performance, lassitude, and weakness. As the disease progresses, there is gradual loss of muscle control, somnolence, coma, and death. In its milder form, the progress of this disease may be extremely slow, and mortality may be correspondingly low.

In contrast, trypanosomiasis due to *T. brucei rhodesiense* is a highly acute disease. The major clinical features are the rapid onset of a high fever with hepatosplenomegaly. Central nervous signs are not dramatic, but some individuals may show evidence of an acute myocarditis (138). Affected individuals suffer from overwhelming parasitemia, and death may occur within a few weeks or months (4). Forms of disease intermediate between the typical Gambian or Rhodesian forms may also be observed.

Clinically, the trypanosomiases of domestic animals resemble the human diseases except that neurological signs are rare (165). The predominant features of these diseases are therefore an intermittent fever, extreme anemia, and lymphatic enlargement with hepatosplenomegaly. A progressive cachexia involving both body fat and muscle is a very significant feature, not least from an economic standpoint. As in humans, the course of these diseases is highly variable, ranging from apparent asymptomatic carriage of trypanosomes to a severe acute disease which may be lethal within a few weeks (4, 138).

Distribution of organisms within the body. The location of trypanosomes within affected animals varies according to the species involved. Thus, trypanosomes of the *T. brucei* group (*T. brucei brucei*, *T. brucei gambiense*, and *T. brucei rhodesiense*) are capable of penetrating the capillary endothelium (52, 99) and of multiplying outside the blood-vascular system. The organisms may therefore be widely distributed and can be encountered in connective-tissue fluid, lymph, cerebrospinal fluid, and aqueous humor (42, 71). In contrast, neither *T. congolense* nor *T. vivax* usually leaves the bloodstream (100, 101, 172a). They lodge preferentially within the microcirculation by attaching to the capillary vascular endothelium (12, 26, 100). Because of this binding, the concentration of these organisms may be 5 to 10 times greater in capillary beds than in large vessels, such as the jugular vein.

Autopsy findings. Both humans and animals which have died of uncomplicated trypanosomiasis show a remarkable absence of gross lesions (100, 138). Indeed, autopsy of cases of the uncomplicated diseases may reveal little more than loss of body fat and a tendency for such organs as the lung, liver, and kidneys to be wet and swollen and for the spleen and lymph nodes to be enlarged (46, 100, 138, 154). After prolonged infections, the spleen, thymus, and lymph nodes may atrophy.

Histological findings. The microscopic lesions of trypanosomiasis are associated with a severe meningoencephalitis in humans (64, 138), with extensive endothelial damage in the microvasculature (44, 46, 52, 77, 100, 125), with structural changes in lymphoid organs, and with a response to severe hemolytic anemia (46, 100).

The cerebral lesions in humans are generally associated with a marked meningeal infiltration and perivascular cuffing by round cells, i.e., lymphocytes, plasma cells and their derivative morular cells, and microglia (112). The microvascular endothelial damage appears to occur as a

result of endothelial denudation associated with local accumulations of trypanosomes (46, 100). Occasionally, this local damage may lead to local perivascular inflammatory cell infiltration (100, 154), to hemorrhage (102), or to edema (44, 53, 100). It may also result in local adherence of leukocytes, in platelet aggregation, and in the development of thrombotic lesions (35, 100, 182). In its most severe forms, disseminated intravascular coagulation may result (13, 20, 61, 79, 153, 183). These microvascular lesions are widely distributed throughout the body but are commonly found in the brain, myocardium, liver, and kidneys (46, 100, 138).

Immunological lesions. Animals infected with trypanosomes develop a number of immunological disorders of varying severity. First, circulating immune complexes may be generated as a result of the persistent antigenemia and resulting immune stimulation (22, 91). The deposition of these complexes on erythrocytes may contribute to the anemia (90, 175), whereas their deposition on glomerular basement membranes may lead to the development of a glomerulonephritis (91, 128, 154). Infected individuals also show a marked depletion of functional T lymphocytes in their spleen and lymph nodes associated with a general disruption of lymphoid tissue structure (46, 74, 100, 125). Serum immunoglobulin levels in infected animals may show significant differences from normal. For example, serum immunoglobulin M (IgM) levels may be greatly elevated (11, 72, 87, 89, 102, 144, 156) rising on occasion to 20 times normal values (89; K. Nielsen, J. Sheppard, I. R. Tizard, and W. L. Holmes, Immunology, in press). Free light chains may also be produced (60). This IgM appears to be directed against a number of antigens. Thus, although some is specifically antitrypanosome (156), the remainder may be heterophil (72, 105), antiglobulin (72), or directed against autoantigens (106, 111, 200). In contrast to the rise in IgM levels, serum IgG tends to remain relatively constant, at values close to normal (89), whereas (in cattle) serum IgA and IgE levels may be severely depressed (Nielsen et al., in press). Catabolic studies indicate that the half-life of all serum immunoglobulin classes is shortened (K. Nielsen, J. Sheppard, W. L. Holmes, and I. R. Tizard, Immunology, in press).

Serum complement levels are also significantly lowered in trypanosomiases (62, 80, 81, 89, 128, 134, 196), the most severely affected components being the early ones, such as C1 and C3 (89, 128, 132; Nielsen, Sheppard, Tizard, and Holmes, Immunology, in press). Properdin levels appear to remain at normal values. This hypocomplementemia may be partially due, at least in experimental animals, to fixation by circulating immune complexes. This is implied by the high levels of serum immunoconglutinins encountered in some infected animals, such as rabbits (78) and sheep (88).

Perhaps the most significant of all the immunological lesions which occur in the trypanosomiases is a profound immunosuppression. This immunosuppression, which involves both the antibody-mediated (51, 54, 75, 76, 84, 98, 181) and the cell-mediated (63, 97, 112) immune systems may be the most important pathogenic mechanism in these diseases. Because of this immunosuppression, it is common for trypanosome-infected individuals, both human and animal, to succumb not directly to the trypanosomes but to secondary infections and die as a result of pneumonias, sepsis, enteritis, or virus infections (4, 100).

The severe anemia observed in all infected species occurs as a result of extravascular hemolysis (83, 109, 154, 191, 195). Its mechanisms are not entirely clear, but some of them probably have an immunological basis. It has been demonstrated that the erythrocytes of infected animals may absorb trypanosome antigens (67, 90, 107, 195) and may then bind antitrypanosome antibody (81, 90). As a result of this, these erythrocytes will be eliminated by the mononuclear-phagocytic system (107, 108, 110, 125, 154).

TRYPANOSOME TOXINS

Historical Concepts

Given the paucity of gross lesions associated with the trypanosomiases, it has not been possible to develop a generally acceptable hypothesis which would account for the progressive illness and death in infected animals. However, the suggestion that trypanosomes could produce "toxins" was an early one. Preliminary evidence for the production of toxic material by trypanosomes was first obtained by Laveran and Mesnil, in 1902 (94). These workers looked for toxic activities in various preparations of killed *T. brucei brucei*. They succeeded in killing a single rat with freeze-thawed *T. brucei brucei* given by the intracardiac route. This animal died 3 h later with rigor and convulsions. On autopsy it had hyperemic meninges but no other lesions. Laveran and Mesnil attempted to reproduce this reaction with desiccated or heat-killed *T. brucei brucei* and even studied the effect of placing collodion sacs filled with *T. brucei brucei*-infected rat blood into the peritoneal cavities of rats but without success. More persuasive evidence for the existence of a trypanosome toxin was obtained by Landsteiner and Raubitschek (92) who studied *T. equiperdum*. (It is possible that this organism was in fact *T. brucei brucei*

[L. Goodwin, personal communication].) These workers demonstrated that if a suspension of these organisms in saline was left overnight in an "ice-box," then the suspension acquired the capacity to lyse erythrocytes. The hemolytic factor was soluble in ethanol, and it was therefore suggested that it might be lipid in nature. However, its precise composition remains unresolved. In spite of the results of Landsteiner and Raubitschek, the attempts of other early workers to demonstrate the presence of toxic factors were only erratically successful. Laveran and Pettit (95) in 1911 showed in a fairly detailed study that subcutaneous or intraperitoneal injection of dried, reconstituted *T. evansi* or *T. brucei brucei* into mice resulted in a drop in body temperature, weakness, convulsions, and loss of reflexes. Except for a very few experiments, in which more than one dose was given and resulted in death, the mice usually recovered 3 to 5 h after injection and showed no changes on autopsy. Although these authors envisaged their active material as an "endotoxin" elaborated by trypanosomes, there is little doubt that this was actually an anaphylatoxin, as judged by its biological activities. The first report of anaphylatoxin production by trypanosomes was published in 1911 by Marcona (114). Marcona succeeded in producing a single fatal anaphylactoid reaction in a guinea pig injected with guinea pig serum "toxified" by incubation with a washed suspension of *T. brucei brucei*. Another publication that year (48) also reported on "trypanotoxins" similar to anaphylatoxins but gave scant details. In a comprehensive and lengthy study, Novy and his colleagues (136) demonstrated anaphylatoxin production by suspensions of five different trypanosome species (*T. evansi*, *T. equinum*, *T. equiperdum*, *T. brucei brucei*, and *T. lewisi*) and showed that anaphylatoxin production did not depend on the viability of the organism. They also demonstrated that the same mass of trypanosomes could be used repeatedly in the generation of anaphylatoxin with either rat or guinea pig sera. Their toxic material was produced within a matter of minutes and appeared to be reasonably stable upon storage. In the same publication, evidence of in vivo anaphylatoxin production by serum transfer experiments was also produced. However, these results were not felt to be conclusive. Novy and his co-workers claimed that the observed effects were not directly due to trypanosomes but rather due to "the disturbance in the colloid state of plasma constituents caused by the alien material." It is still by no means clear whether anaphylatoxin production in vivo contributes significantly to the pathogenesis of trypanosomiasis. However, it is possible to demonstrate that trypanosomes can activate complement (see below), and it may be shown that histamine and kinin levels do increase in the urine and tissues of animals acutely infected with *T. brucei brucei* (16, 19, 23, 147, 184, 197).

Because of the early failures to demonstrate conclusively the existence of trypanosome toxins, the subject fell into disrepute, to be revived briefly by an observation of Fiennes (45). During the course of a study of trypanosomiasis in adult cattle due to *T. congolense*, Fiennes was able to demonstrate, in one animal, the intermittent presence of a plasma factor capable of lysing normal bovine erythrocytes. A similar phenomenon has recently been reported to occur in mice infected with *T. brucei brucei*. The serum of these animals appears to be capable of modifying normal mice erythrocytes in such a way that their life-span is reduced when washed and inoculated into syngeneic recipients (77). Neither in this case nor in the bovine case of Fiennes was the offending factor isolated or characterized.

Thus, until recently, the failure of most workers to demonstrate trypanotoxin production led most investigators to deny its existence. Nevertheless, evidence has gradually accumulated to suggest not only that living trypanosomes may release factors with significant biological activity in vivo but that on their death and dissolution they may also generate highly active autolytic products.

Factors Released by Living Trypanosomes

Trypanosome surface components. The pathogenic African trypanosomes possess, when circulating in the bloodstream, a thick (12 to 15 nm) surface coat of electron-dense material. This coat consists of glycoprotein of about 64,000 daltons. It is antigenic and stimulates an immune response. Trypanosomes can, however, shed this cost and replace it with another antigenically dissimilar glycoprotein (2, 3, 34, 36, 189). This process may be repeated, apparently indefinitely. In this way the organisms may avoid the consequences of the host response and persist within the bloodstream. The shed cell surface glycoprotein may be detected in plasma, where it is known as exoantigen. Exoantigen has a number of biological activities. First, it is antigenic and may stimulate a protective immune response (67, 68). When free in plasma, it is presumably capable of combining with antibody, so forming immune complexes. It may therefore contribute to the immune-complex-derived lesions observed in trypanosomiasis (22, 91). It can also bind directly to erythrocytes (107, 195, 200), in which case it may provoke their sensitization

by antitrypanosome antibodies and result in their accelerated clearance.

An alternative mechanism of erythrocyte destruction may occur through direct complement activation on the erythrocyte surface in the absence of antibody. The surface glycoproteins of *T. brucei brucei* are capable of activating the classical complement pathway through C1, C4, and C2 (127). Since these glycoproteins can bind to erythrocytes, they may activate complement in this location and so provoke significant erythrocyte destruction. This suggestion is supported by the detection of bovine C3 on the surface of erythrocytes of anemic *T. congolense*-infected calves in the absence of immunoglobulin (90). This also supports the theories of workers who have suggested that exoantigens may function as hemolysins (34, 37) on the doubtful basis of the direct relationship which may be observed between the severity of the anemia in infected animals and the number of circulating trypanosomes.

It may also be shown that purified cell surface glycoprotein will cause extravasation of capillary fluid on intradermal injection into rats and that this reaction may be inhibited by pretreatment with antihistamines (127). This phenomenon is probably mediated through anaphylatoxin generation.

A second form of trypanosome secretion which occurs in vivo is the production of secretory filaments (39, 198). These filaments have been reported to be produced by stumpy forms of *T. brucei brucei*. They appear to be secreted from the trypanosome via the flagellar pocket, becoming surrounded during the process by a surface glycoprotein coat. They originate in the region of the golgi apparatus and contain acid phosphatase activity. Although the biological significance of this is unclear, it is evident that this process provides an alternative mechanism by which the trypanosome surface glycoprotein may be shed into the plasma.

Trypanosome anesthetics. (i) Synthesis and biochemistry. African trypanosomes can catabolize tryptophan, tyrosine, and phenylalanine, forming compounds which may contribute to pathology characteristic of African trypanosomiasis (166, 168, 169). *T. brucei gambiense* can metabolize tryptophan to indole-3-ethanol (tryptophol, TOL), indole-3-acetic acid (IAA), and indole-3-lactic acid (ILA) and 5-hydroxytryptophan to 5-hydroxytryptophol (169).

Trypanosome tryptophan metabolism to TOL, IAA, and ILA occurs via the classical Ehrlich pathway (Fig. 1). Tryptophan can be transaminated to indole-3-pyruvic acid, with α-ketoglutarate or oxaloacetate used as the amine group acceptor. Pyridoxal phosphate may not be required for tryptophan transamination, although this has not been rigorously examined. In the presence of excess reduced nicotinamide adenine dinucleotide (NAD), ILA is the predominant indole product. Reduction of the indole pyruvate may be catalyzed by an aromatic lactate dehydrogenase. In the absence of excess exogenous reduced NAD, TOL, IAA, and ILA are formed in approximately equimolar concentrations. Indole pyruvate is first decarboxylated and then reduced to TOL or oxidized to IAA (169). IAA and ILA can be rapidly eliminated from mammalian systems by urinary excretion (6, 191). TOL injected into mammals is rapidly converted to IAA and excreted (158).

The significance of this catabolic pathway to the trypanosome is not known. Tryptophan catabolism has been suggested to be important in maintaining the oxidation-reduction balance of the trypanosome. In trypanosomes found in the bloodstream which lack terminal cytochrome systems, reductive reactions to form ILA and TOL may help regenerate oxidized NAD (168). It has been suggested that aromatic amino acid transamination is coupled to the trypanosome glycolytic pathway through the amination of pyruvate, thus detoxifying pyruvate while supplying the amino acid alanine. Indole acids subsequently formed could be readily excreted in the host's urine. Alanine is known to be markedly increased in the blood of infected animals, whereas tryptophan and tyrosine are greatly reduced (129). Recent results, however, indicate that pyruvate cannot act as the direct amine acceptor for tryptophan or tyrosine transamination (S. C. Merritt, J. E. Hall, and J. R. Seed, unpublished data), although pyruvate may be linked indirectly via other transaminations. The role of transaminase reactions in trypanosome metabolism needs to be examined more closely.

(ii) Toxic biological properties. The major interest in tryptophan metabolism by the trypanosome has been due to the involvement of the indoles in production of a sleeplike or comatose state and in alteration of body temperature. TOL, injected intraperitoneally into mice, rats, and chicks, has been shown to produce a sleeplike behavioral state (158, 170; J. R. Seed, T. M. Seed, and J. Sechelski, Comp. Biochem. Physiol., in press). In the cat, it has been found that the electroencephalogram patterns after TOL injection resemble not true sleep but rather a state of wakefulness with immobilization, a state more closely resembling coma or paralysis (123). In addition, TOL has been shown to lower body temperature (43, 158), and it can also suppress the humoral immune response to mice

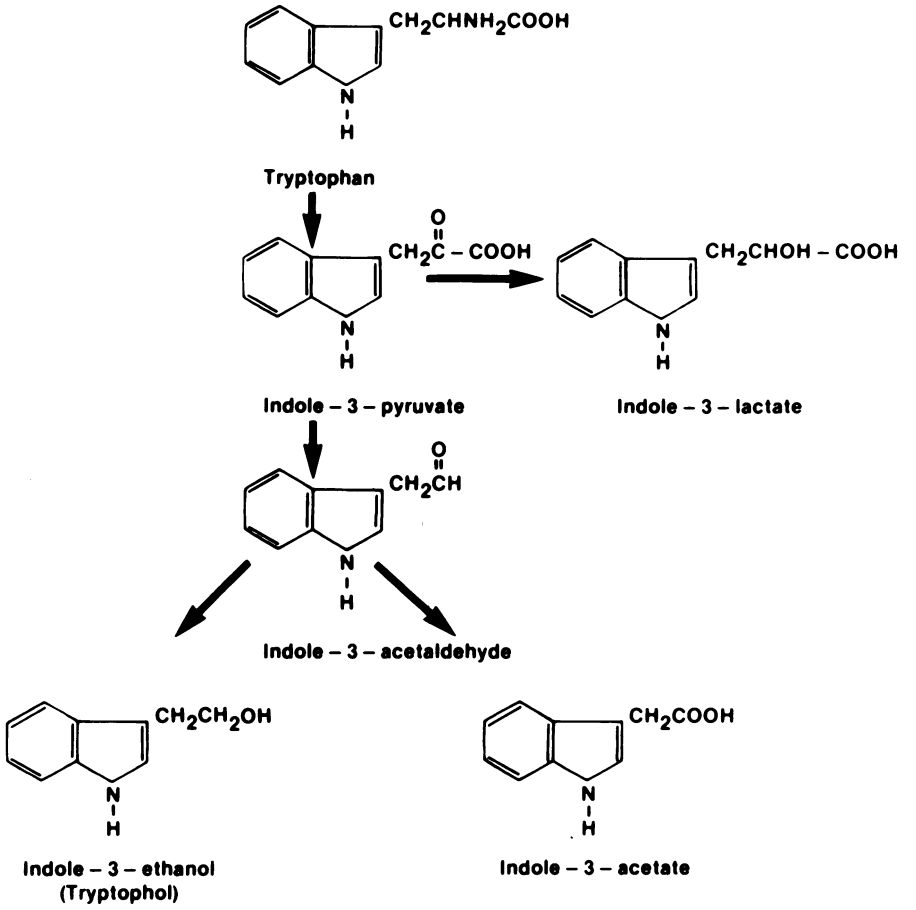

FIG. 1. *T. brucei gambiense tryptophan metabolic pathway.*

after intraperitoneal injection (1). The sleeplike behavioral state, the change in body temperature, and the immunosuppression produced by TOL all resemble clinical symptoms observed in humans chronically infected with African trypanosomes. It has therefore been hypothesized that TOL is a metabolic toxin produced by the African trypanosomes.

TOL may exert its toxic effects by acting on cell membranes, perhaps by combining with the outer lipid bilayer in a manner similar to that reported for the structurally related compounds indole and 3-methylindole (25; Seed et al., Comp. Biochem. Physiol., in press). The interaction of TOL with cell membranes has been investigated by using a human erythrocyte model. TOL causes erythrocyte lysis, with the interaction between TOL and the membrane being rapid and irreversible. It has also been demonstrated that TOL will produce in vitro changes in the oxygen consumption of Ehrlich ascites tumor cells at concentrations considerably lower than those required to produce sleep and temperature changes in vivo (J. E. Hall, personal observation). It has therefore been hypothesized that trypanosomes within extravascular sites of the central nervous system produce sufficient quantities of TOL to alter the permeability of synaptic membranes and thereby cause changes in the transmission of nerve impulses, resulting in behavioral changes as well as other systemic effects, such as immunosuppression. Similar modes of action on the cell membrane have been hypothesized for a variety of anesthetics.

To determine if TOL is indeed a trypanosome-produced toxin, the exact quantities produced in vitro and in vivo must be measured. Ultimately, it will be necessary to correlate blood and cerebrospinal fluid concentrations of TOL with the various behavioral states observed during trypanosomiasis.

(iii) Quantity in vivo. The minimal amounts of TOL, IAA, and ILA produced by a known number of trypanosomes in vitro have been determined by thin-layer chromatography for the separation and isolation of these compounds

followed by their quantitation by gas chromatography. The amounts produced are 167 pg of TOL, 170 pg of IAA, and 184 pg of ILA per 10^8 blood trypanosomes per h. Using these figures, plus the trypanosome generation time and an estimate of the total number of parasites at peak parasitemia, has allowed a minimal estimate of the total amount of TOL produced in an infected mouse before death to be made. Using these figures, it has been estimated that a minimum of 3.2 mg of TOL per kg of body weight could be formed in an infected mouse, along with similar concentrations of IAA and ILA. Currently, the actual amounts of these compounds in the urine and sera of infected mice are being investigated. Until these data are available, it can only be stated that the calculated amount of TOL produced in vivo is low but still consistent with the hypothesis that this compound would produce behavioral changes and neuropathology. It has been recently shown that the amount of ILA is increased in infected mice (J. E. Hall and J. R. Seed, unpublished data).

The African trypanosomes can therefore synthesize potentially toxic indole and catechol metabolites which, at pharmacological doses, produce a number of the clinical symptoms observed in humans. The alterations in serum (and brain) amino acid levels, the presence of at least one potentially toxic metabolite in the urine of infected animals, and the reaction of TOL with the cell membrane are all consistent with the hypothesis presented. Substantiation of the hypothesis, however, will require in vivo quantitation and the correlation of concentrations with specific behavioral stages, such as lethargy and neurophysiological changes.

(iv) Other potentially toxic metabolites. Although research has primarily concentrated on indole metabolism by the trypanosomes and the production of toxic indoles, it should again be noted that both tyrosine and phenylalanine are also catabolized to other potentially toxic compounds, i.e., *p*-hydroxyphenylethanol and phenylpyruvate, respectively. For example, it is believed that phenylpyruvate is at least in part responsible for the symptoms observed in phenylketonuria (190). It has been tentatively demonstrated that increased levels of *p*-hydroxyphenylpyruvate are found in the urine of chronically infected animals (167).

Intravascular trypanosomes are engaged in ceaseless motion, a motion which involves the lavish expenditure of energy. The source of this energy is blood glucose, which is utilized by the trypanosomes, eventually yielding large quantities of pyruvate for prolonged periods (24, 58, 188). Persistent high levels of pyruvate may exert some toxic effects on cells (54, 64). In *T. brucei brucei* infections, for example, it has been shown that the high pyruvate levels in tissue fluid may be toxic for fibroblasts and perhaps contribute to the breakdown in connective-tissue structure observed (55, 56, 153). The pyruvate in the bloodstream is efficiently buffered in healthy animals, but animals may become severely acidotic in the terminal stages of these diseases (28, 53).

Factors Released from Dead or Dying Trypanosomes

Clinical observation suggests that exacerbations of clinical trypanosomiasis may be related to the occurrence of intravascular destruction of the organisms. For example, infected animals appear to sicken and occasionally suffer a severe hemolytic crisis at the time when the parasitemia is falling rapidly as a result of immune trypanolysis (48, 90, 195). Similarly, a shock syndrome similar to a Jarisch-Herxheimer reaction is a characteristic feature of the response of individuals after treatment with trypanocidal drugs at a time when they have a high parasitemia (138; P. de Raadt, Abstr. 13th Int. Congr. Trop. Med. Malaria, Tehran, 1968, p. 336–337), and early in the infection peaks of fever appear to be associated with the relapses (159). It is therefore not unreasonable to suggest that the intravascular destruction of trypanosomes may lead to the release of intracellular components, some of which may be biologically active and possibly also toxic. Since the trypanosome parasitemia shows cyclical fluctuations as a result of the immune destruction of immunologically distinct trypanosome subpopulations, a feature of these diseases is the prolonged exposure of infected animals to dead, dying, and disrupted trypanosomes. In this way, animals may be exposed to trypanosome contents, including a variety of enzymes as well as the products of enzymic degradation.

Trypanosome enzymes. (i) Proteases. Trypanosomes possess lysosomes which contain both acid phosphatase and cathepsin D (185). These enzymes are released on autolysis, and the cathepsin D together with other proteases may act on a number of biologically significant substrates. Thus, proteases acting on plasminogen generate plasmin, and rabbits infected with *T. brucei brucei* show a marked increase in their serum levels of both plasmin and fibrinogen degradation products (20). Proteases are also capable of acting on kininogens, although ultrasonically treated *T. brucei brucei* has been reported to contain no kinin-forming enzymes (18). Nevertheless, kinins are generated in large amounts in several forms of trypanosomiasis (16, 146, 184), and this generation is associated with

trypanolysis in vivo (17, 19, 21, 22). Notwithstanding the failure to demonstrate kininogenases in trypanosomes, it is known that cathepsin D can act on leukokininogen to generate leukokinins (59).

Complement-activating material from *T. lewisi* and *T. congolense* thought to contain proteases has been described by Nielsen and his colleagues (130–133). This material was released from ultrasonically treated trypanosome suspensions. It was inactivated by phenylmethylsulfonylfluoride, by trichloroacetic acid, and by heating (100°C for 15 min). Since this material accounted for less than 10% of the overall anticomplementary activity of these trypanosome preparations, it was not considered by these authors to be of major significance. Nevertheless, the existence of this activity demonstrates the potential of dead trypanosomes for consuming serum complement and possibly, therefore, for generating anaphylatoxins.

It may also be pointed out that active proteolytic enzymes acting in a microvascular location are potentially capable of causing local endothelial damage and changes in vascular permeability through direct effects on nearby cells. These proteases could also be responsible for the vascular permeability factor demonstrated in extracts of *T. brucei gambiense* (155).

(ii) Phospholipases. A second group of enzymes which appear to be generated by autolysed trypanosomes are the phospholipases and lysophospholipases. Autolysing trypanosomes do not appear to generate lipases (176).

Both *T. brucei brucei* and *T. congolense* are, when freshly isolated, relatively poor in phospholipase activity. However, on autolysis for 8 to 24 h at 20°C, this activity increases greatly (177). In *T. congolense*, the phospholipase activity may reach 40 times the level in fresh organisms by 24 h. This generation of phospholipase activity is unaffected by sonic treatment of the organisms immediately after isolation, but it is disproportionately inhibited by dilution of the trypanosome suspension, thus implying that some form of autocatalytic process is involved in its generation. The phospholipase of *T. congolense* appears capable of hydrolyzing phosphatidylcholine (lecithin) only at the 1-acyl position and is therefore a phospholipase A_1 (177). It is highly active, having a K_m of 1 mM for phosphatidylcholine substrates (A. Mellors, unpublished data). *T. congolense* is also rich in lysophospholipase activity when freshly isolated. It is probable that this serves to prevent the in vivo accumulation of lysophosphatidylcholine, which is membrane active and thus potentially toxic.

T. brucei brucei generates phospholipase A_1 activity on autolysis in a similar manner to that of *T. congolense*.

The nonpathogenic rat trypanosome *T. lewisi* has phospholipase levels in freshly isolated organisms similar to those found in fresh *T. brucei brucei* and fresh *T. congolense*, but these enzymes fail to increase their activity even on prolonged autolysis (176). The function(s) of these trypanosome phospholipases is unclear. However, the salivarian trypanosomes undertake extensive mitochondrial membrane reorganization on being ingested by tsetse flies (186), and it is possible that the phospholipases are required to play a role in this process by breaking down membrane-associated phospholipids.

Phospholipases possess a variety of significant biological activities that are of relevance to the lesions observed in the trypanosomiases (Fig. 2). For example, phospholipase A_1 acts on trypanosome phosphatidylcholine to yield free fatty acids (FFAs) and lysophosphatidylcholine. The lysophosphatidylcholine is then further degraded by the lysophospholipase to yield more FFAs and glycerophosphorylcholine (177). The FFAs generated in this way are probably of major significance in the pathogenesis of trypanosomiasis and are discussed more fully in a later section.

Phospholipases themselves may exert a direct biological effect on an animal, since they can destroy cell membrane phospholipids. As a result, they may be both cytotoxic and hemolytic (29). In general, it appears that the phospholipase A_1 is, by itself, poorly lytic. However, in conjunction with detergent-like molecules, such as FFAs, it is capable of significant cell membrane disruption (30). Because *T. congolense* tends to locate within the microvasculature, this enzyme may provoke endothelial damage (27) and, possibly, also some erythrocyte destruction. In the case of *T. brucei brucei*, autolysis within connective tissues may result in toxic changes in fibroblasts, breakdown of connective tissue, and the development of widespread edema (52). Injection of trypanosome preparations containing such enzymes could lead to an increase in vascular permeability and the development of a local inflammatory response. Such a reaction is observed after intradermal inoculation of *T. brucei gambiense* (155), *T. brucei brucei* (127), and *T. congolense* (173).

Phospholipase activity has been associated with the pathogenicity of certain strains of amoebae, such as *Acanthamoeba* (187). Trypanosome phospholipases are also capable, in subhemolytic doses, of degranulating mast cells both in vivo and in vitro (I. R. Tizard, unpublished data). This process and the subsequent

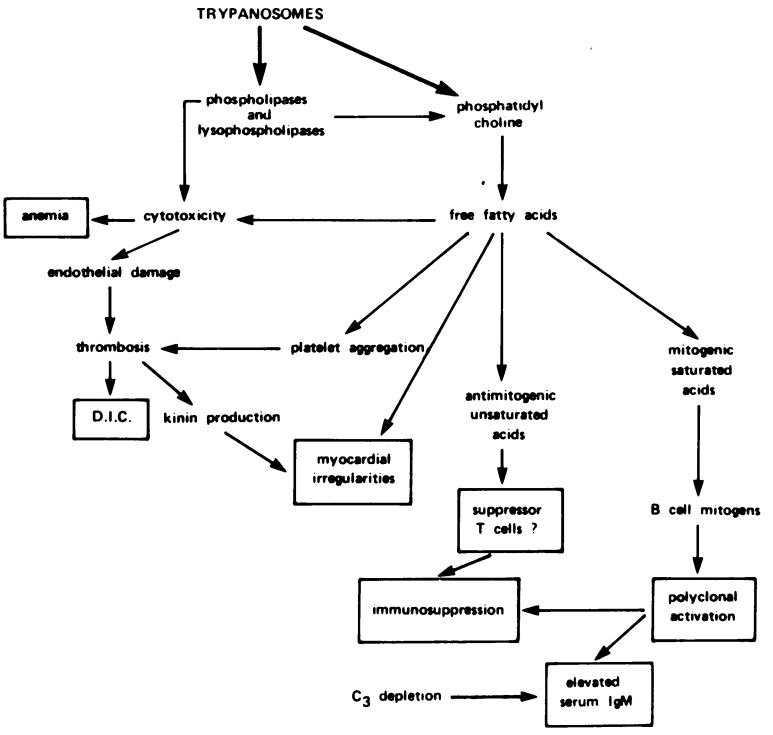

FIG. 2. *Possible roles of trypanosome phospholipases and FFAs in the pathogenesis of the trypanosomiases.*

release of vasoactive agents may lead not only to local changes in vascular permeability but also to an elevation in blood kinins and to the development of an anaphylactoid syndrome. Both of these phenomena are observed in trypanosomiasis (16, 19, 21, 23, 147, 184, 197).

It is possible that the lysophospholipase activity generated by autolysed *T. congolense* is also of some pathological significance. Thus, in cattle infected with *T. congolense*, the serum lysophosphatidylcholine drops from 7 to 10% of blood lipids to 0 to 3% (148–150). Since lysophosphatidylcholine is required for the utilization of cholesterol and possibly other lipids (151), its absence may result in blocking of lipid utilization. As a result of this blockage, animals may mobilize their body fats. A remarkable loss of body fat is a characteristic feature of trypanosomiasis in both humans and animals (100).

Trypanosome lipids. (i) Lipopolysaccharides. The trypanosome responsible for human trypanosomiasis in South America, *T. cruzi*, contains a biologically active lipopolysaccharide known as chagastoxin (57, 86). There is no evidence to suggest that similar material is present in the African trypanosomes. Chagastoxin, when given to mice, causes a focal necrosis and round-cell infiltration of the liver, kidneys, and heart. It also renders animals more susceptible to *T. cruzi*. In contrast to the bacterial lipopolysaccharides, it does not activate complement or elicit a Shwartzman reaction, nor does it induce a significantly protective immune response (86, 160).

Nielsen and Sheppard (130) and Nielsen et al. (131–133; Nielsen, Sheppard, Tizard, and Holmes, Immunology, in press) have demonstrated that both *T. congolense* and *T. lewisi* contain a factor which is possibly lipopolysaccharide in nature and which activates complement both in vivo and in vitro. This material is apparently not exposed in the cell walls of trypanosomes, since living organisms of these species are not anticomplementary. However, ageing or mechanical disruption of either of these species liberates large amounts of a complement-fixing substance. This material may be subjected to treatment with 10% (final concentration) trichloroacetic acid with very little loss of activity. Gel filtration of this crude preparation indicates that the active substance is of heterogeneous molecular size, since anticomplementary activity may be found throughout the elution spectrum. Ion-exchange chromatography, however, reveals a homogeneously charged population of molecules, as most of the activity can be eluted in a single peak. Polyacrylamide gel electrophoresis reveals a single, clear, well-defined band as

stained with Alcian Blue. The purified factor contains hexoses. This complement-activating factor (CAF-T) is resistant to acid and alkaline hydrolysis and to a temperature of 100°C for 15 min. It is unaffected by inhibitors of proteolytic enzymes and is soluble, to a variable extent, in fat solvents. CAF-T is capable of inactivating bovine, human, guinea pig, and rat complements and appears to act by blocking the classical pathway at the C1s stage (J. Minta, personal communication). This trypanosome-derived, complement-activating polysaccharide is not unique. A number of other polysaccharides derived from other sources have been shown in the past to activate complement. Although most of these act through the alternate pathway (115, 126, 142, 171, 194), some have been reported to activate C1 (14, 15). The variant-specific glycoprotein of trypanosomes also appears to act through the classical pathway (127).

(ii) Free fatty acids. When freshly isolated trypanosomes, such as *T. congolense*, are mixed with washed sheep erythrocytes, no visible reaction occurs for several hours. By about 9 to 10 h, however, the erythrocytes commence to lyse (173). This is the phenomenon first described by Landsteiner and Raubitschek (92). On analysis, it may be shown that the hemolytic material consists of FFAs generated by the action of the trypanosome phospholipases on endogenous phosphatidylcholine (173, 176). If any lysophosphatidylcholine is also present in the autolysate, it too may contribute to the hemolytic process (179). A similar phenomenon also occurs with *T. brucei brucei* (179).

Closer examination of the autolytic process indicates that the concentration of FFAs in a suspension of *T. congolense* held at 20°C rises steadily but that the concentration required for hemolysis is only reached after 9 to 10 h, by which time 3×10^9 organisms have generated 0.01 to 0.03 mg of FFA (172). Gas chromatography of the purified trypanosome FFAs indicates that they are a mixture consisting largely of palmitic, stearic, and linoleic acids (each constituting about 25% of the total FFA), with oleic, γ-linolenic, and arachidic acids present in lesser amounts. On testing individual fatty acids, it is clear that linoleic acid is primarily responsible for the hemolytic properties of the mixture (175).

FFAs are hemolytic and cytotoxic as a result of their detergent-like properties. They dissolve in the lipid component of cell membranes. At low concentrations this may result in membrane stabilization, whereas at higher concentrations they destabilize the membrane and so cause lysis (10). All cells appear to be equally susceptible to this lytic action. However, since these acids were first detected by a hemolytic assay and since anemia is so important in the pathogenesis of trypanosomiasis, it was necessary to determine whether these fatty acids contributed to hemolysis in vivo in a similar manner to that observed in blackwater fever (93). Further investigations showed that the hemolytic properties of these trypanosome-derived FFAs were probably not significant in vivo, at least not in cattle infected with *T. congolense* (172). The major reason for this is that FFAs are rapidly bound to serum albumin in vivo and cannot cause hemolysis when in the bound state (50, 163, 164). In the case of the *T. congolense*-derived fatty acids, the evidence for their lack of significance is as follows. First, small quantities of serum albumin can completely block hemolysis in vitro (175). Second, the amount of free fatty acid calculated to be released by the trypanosomes in vivo is at least two orders of magnitude less than that required to saturate the albumin and enable hemolytic fatty acids to exist free in plasma. Third, pretreatment of bovine erythrocytes with subhemolytic doses of FFA in vitro has no effect on their subsequent survival in vivo (175). Finally, there is no evidence that the anemia which occurs in *T. congolense* infections in cattle is mediated by an intravascular hemolysis (46, 98).

Although it is clear that FFAs are not a significant cause of erythrocyte destruction in cattle infected with *T. congolense*, it is possible that they may be effective in this respect in other infected animals. In rats infected with *T. congolense*, for example, the parasitemia is considerably higher than that observed in cattle, and it is theoretically possible that dying trypanosomes may, in this species, release sufficient FFA to saturate the albumin and lyse erythrocytes in vivo (175).

FFAs are capable of inducing thrombosis (31, 70), lipid accumulation in cultured cells (124), myocardial lesions (193), and thrombocytopenia (161, 199) in experimental animals. All of these lesions have been reported to occur in trypanosomiasis, but there is evidence presently available which would prove that any of these lesions are due to the activation of trypanosome-derived FFAs.

FFAs are also highly active stimulators of insulin secretion (33) and as a result may induce a hypoglycemia. This, too, has been reported to contribute to the deaths of trypanosome-infected animals (69). However, as with many of the other features of fatty acid-induced toxicity, it has not been experimentally demonstrated that trypanosome fatty acids contribute to the hypoglycemia observed in infected animals.

Although FFAs bound to serum albumin are no longer cytolytic, they remain capable of modifying other cellular functions (5). For example,

certain FFAs, such as margaric ($C_{17:0}$), linoleic ($C_{18:2}$), γ-linolenic ($C_{18\omega3}$), and arachidonic ($C_{20:4}$) acids, have been shown to be capable of suppressing immune responses. Of these acids, linoleic acid, which constitutes 22.5% of the FFA released from *T. congolense* and 23.3% of that released from *T. brucei brucei*, has been most extensively studied. It can prolong skin allograft survival in mice (117, 118, 146), and oral administration of a mixture of linoleic and linolenic acids to human allograft recipients has been shown to be of benefit in delaying the onset and reducing the severity of rejection episodes (180). When mice are given a daily dose of 0.1 mg of an FFA mixture (a dose equivalent to the amount of FFA generated by 4.4×10^9 organisms) of similar composition to that found in *T. congolense*, this significantly suppresses the immune response of the animals, as reflected in the development of plaque-forming cells to sheep erythrocytes (Tizard, unpublished data).

The immunosuppressive effects of fatty acids appear to be due to a direct action on lymphocytes. Thus, in the presence of linoleic acid, the response of lymphocytes to phytohemagglutinin, purified protein derivative of tuberculin, and antigen is reduced (117–119, 121, 192). Trypanosome suspensions have also been shown to inhibit the response of mouse lymphocytes to phytohemagglutinin (8, 97). Linoleic acid given to mice or rats has been shown to disrupt the architecture of the spleen and lymph nodes, decreasing the proportion of white pulp and its uptake of tritiated uridine (116). An identical lesion is observed in trypanosomiasis (46, 74, 100, 125), and, if prolonged, its outcome is the same, namely, the production of destructive changes and splenic atrophy. Since the immunosuppressive effect of linoleic acid in vivo is prevented by splenectomy (116, 120), it has been suggested that a suppressor cell population may be involved in these processes. Suppressor cells have also been shown to be active in trypanosomiasis (32, 38, 82).

There are several possible mechanisms by which fatty acids may induce immunosuppression. These include membrane perturbation as a result of insertion of fatty acid molecules into cell membranes (10). At low concentrations, perturbation would tend to reduce membrane fluidity and consequently inhibit cell division (146).

A second potential mechanism by which fatty acids may regulate the immune responses is through their role as prostaglandin precursors. Some of these acids, notably linoleic acid, are rapidly converted to prostaglandins within cell membranes (47, 120). These intramembranous prostaglandins control lymphocyte reactivity either through an action on calcium binding or by regulating cyclic nucleotide levels (66, 96, 122, 143). Indeed, FFAs can activate guanylate cyclase (7) and either inhibit (41) or enhance adenylate cyclase activity (137). Stimulation of adenylate cyclase and a subsequent increase in the intracellular concentration of cyclic 3′,5′-adenosine monophosphate will reduce lymphocyte reactivity, whereas stimulation of guanylate cyclase will have the reverse effect (24).

Toxic Factors of Unknown Origin

Trypanosome hemolysins. It was previously noted that Fiennes (45) demonstrated the presence of a hemolysin in the serum of a single *T. congolense*-infected cow. Unfortunately, it has not proved possible to repeat this observation, and the hemolysin has not been characterized. However, Huan (73) reported that a slow-acting hemolytic activity was generated in suspensions of *T. brucei brucei*, *T. brucei gambiense*, *T. vivax*, and *T. congolense*. These suspensions were found capable of causing a slow lysis of sheep erythrocytes in vitro. The hemolytic activity was fractionated on G-100 and G-25 Sephadex, and it was claimed that it was associated with a protein with a molecular weight of about 10,000. Unfortunately, the published data do not make clear for how long the trypanosomes must be incubated before this material is generated or their viability during the incubation period. It is, therefore, not possible to determine whether this material is generated by living trypanosomes or is an autolytic product. Until full details of the preparation of this material are published and the work is repeated by other investigators, the significance of this factor cannot be determined.

Trypanosome mitogens. Esuruoso was the first to demonstrate (40) that homogenized *T. brucei brucei* suspensions were mitogenic for the spleen cells of both normal and nu/nu mice, and he suggested that some component of these organisms served as a B-cell mitogen. This conclusion was supported by the failure of spleen cells from cyclophosphamide-treated animals to respond to this same material. Mansfield and his colleagues (110) investigated the mitogenic properties of extracts of *T. congolense* on the spleen cells of mice, rats, and guinea pigs. They were unable to demonstrate any effect on the mouse spleen cells but did show that the material was capable of stimulating rabbit cells. The responding cells in the rabbit were probably unresponsive to phytohemagglutinin, which is a finding compatible with the concept of this material as a B-cell mitogen.

It is difficult to understand Mansfield's failure to demonstrate a mitogenic effect of *T. congo-*

lense on mouse cells, since Assoku and Tizard (8) were able to demonstrate a mouse spleen cell mitogen in autolysates of this organism. These autolysates could stimulate mitosis in the spleen cells of nu/nu mice, and the reaction failed to occur when tested on cells from cyclophosphamide-treated animals. Preliminary studies on the characterization of this mitogen indicated that it was extractable in chloroform-methanol and appeared to consist of a mixture of saturated fatty acids (R. K. G. Assoku, C. A. Hazlett, and I. R. Tizard, Int. Arch. Allergy Appl. Immunol., in press). Like other mitogens, this material was only active over a rather narrow dose range, and in high doses it served to inhibit lymphocyte mitogenicity as shown by the lymphocyte response to bacterial lipopolysaccharide (8). This inhibition may have been due to the direct cytotoxic effect of the FFAs in the trypanosome autolysate.

In contrast to the relatively potent mitogenic activity of *T. congolense* autolysates, similar preparations from the nonpathogenic trypanosomes *T. lewisi* (8) and *T. musculi* (C. A. Hazlett and I. R. Tizard, Clin. Exp. Immunol., in press) are only weakly so. This may be a reflection of the failure of these organisms to generate fatty acids on autolysis. The finding that trypanosomes possess B-cell mitogens lends support to the hypothesis that these organisms function as polyclonal B-cell stimulators (9, 106; Hazlett and Tizard, in press) and that it is this stimulation, in conjunction with a failure in the IgM-to-IgG switch, which accounts for the elevation in serum IgM levels in these diseases (139, 141).

Trypanosome inflammatory factors. Local skin reactions occur at the sites of tsetse fly bites. This trypanosomal "chancre" is a hard, painful, red nodule. On puncture, a clear fluid containing trypanosomes may be withdrawn, and trypanosomes may be shown to be developing within the lesion (103). A typical chancre also occurs when blood forms of trypanosomes are injected intradermally (113), so tsetse fly saliva is not essential for its development.

Some of the factors already described, such as the proteases and phospholipases, are capable of inducing this inflammation. The FFAs of *T. congolense* probably contribute to the mild inflammatory response observed on intradermal inoculation of this organism, whereas the cell surface glycoprotein of *T. brucei brucei*, by activating local complement components, also causes acute inflammation (127).

A vascular-permeability-increasing factor has been extracted from *T. brucei gambiense*. This material was protein in nature and was capable of increasing vascular permeability when injected intradermally into both guinea pigs and rabbits (55). The relationship of this material to the other factors described is unknown. It is quite possible that, as previously noted, these authors could have been looking at protease activity. It would be of considerable interest to determine if protease inhibitors could block these reactions. When rabbits are injected with *T. brucei brucei*, they develop edematous subcutaneous plaques. These plaques are most obvious around the eyes, ears, and scrotum of infected animals (52, 100). It was pointed out several years ago that these lesions resembled those seen in acute serum sickness of rabbits (R. R. A. Coombs, cited in reference 53). In serum sickness, the lesions arise as a result of the activation of complement by immune complexes and the subsequent release of neutrophil proteases into the tissues. It is therefore likely that identical lesions can be induced by organisms capable both of activating complement and of releasing proteases into connective-tissue spaces.

If Millipore diffusion chambers containing 10^6 living or distilled-water-lysed *T. congolense* are implanted intraperitoneally into rats, they provoke a local inflammatory reaction which culminates in the walling-off of the chamber by granulation tissue (174, 178). Large numbers of trypanosomes are required to provoke this reaction, since 10^4 organisms produce an inconstant response, whereas 10^2 organisms are ineffective. The reaction also appears to be somewhat species specific, since chambers containing 10^6 *T. lewisi* or *T. brucei brucei* were generally ineffective. The active material was relatively small, being capable of passing through an ultrafilter with a stated cutoff of 1,000 daltons. It was not generated by trypsinized trypanosomes or by Formol-treated organisms and could not be detected in the serum of rats infected with *T. congolense*. The reaction could not be produced by placing a suspension of FFAs within the chambers. Consequently, its precise nature remains unknown.

Trypanosome hepatotoxins. In 1927 Regendanz and Tropp (145) investigated the blood sugar levels in rats infected by a variety of trypanosome species. They demonstrated that these animals were hypoglycemic at the terminal stages of infection. However, on investigating the liver glycogen, they found it to be depleted but not exhausted. Thus, they argued that the hypoglycemia could not be caused solely by trypanosomes utilizing glucose but that there must also exist some block in the utilization of liver glycogen. They postulated that this might have been due to a trypanosome toxin.

No further work has been undertaken that would directly support or refute this theory, yet

there is evidence for significant hepatic dysfunction in these diseases. For example, jaundice is an occasional feature of some cases of Rhodesian sleeping sickness. Other dysfunctions include an abnormal bromsulphthalein tolerance test, excess urinary urobilinogen, abnormal hippuric acid synthesis, seroflocculation abnormalities (49, 152), and a very marked hypoalbuminemia (138). Finally, the changes in the cytopathology of hepatocytes as well as changes in hepatic enzymes are consistent with the concept of a trypanosome hepatotoxin (104).

Seed and Gam (157) described the existence of liver autoantibody in experimental *T. brucei gambiense* infection and suggested that either this autoantibody or a trypanosome toxin could contribute to the development of hepatic lesions. In 1958, Kawamitsu (85) showed that injection of *T. brucei gambiense* emulsions into normal rats would produce a hypoglycemia and decrease liver glycogen, but this observation has not been repeated.

PATHOGENESIS OF TRYPANOSOMIASIS

Given the wide variety of biologically active factors which may be derived from the African trypanosomes, it is possible to establish a number of potential pathways through which the major lesions of trypanosomiasis may be derived. These major lesions, which are common to most forms of these diseases, include a profound immunosuppression, hypocomplementemia, widespread microvascular damage, and erythrocyte destruction. Factors have been described that are capable of inducing all these lesions. Thus, immunosuppression may be induced by FFAs or by TOL (1). The hypocomplementemia induced by CAF-T, by variant-specific glycoprotein, by proteases, and possibly through immune-complex formation, may also lead to functional immunosuppression. Thus, Nielsen et al. (134) demonstrated that rats decomplemented by CAF-T were rendered highly susceptible to salmonellosis. The hypocomplementemia, by preventing the switch from IgM production to IgG production (140), may also account for the preponderance of IgM synthesis in these infections (9, 135) (Fig. 3).

Microvascular damage, as discussed earlier, may be mediated by phospholipases, perhaps by proteases, by FFAs, and possibly by other uncharacterized factors. It should also be pointed out that biologically active peptides (kinins) are generated in trypanosome-infected animals (19, 21, 23, 122, 147, 184, 197), and these will also serve to increase greatly vascular permeability. Although there is good evidence to suggest that these kinins are generated through complement activation subsequent to immune-complex formation (17, 18, 21, 162), it is not unlikely that an equally important mechanism involves the direct activation of complement by factors such as the variant-specific glycoprotein (127).

Although erythrocyte destruction has been shown to be, at least in part, mediated by immunological mechanisms (90), there exist a number of alternative hemolytic mechanisms. Examples of these include the hemolytic factors of Fiennes (45) and of Huan (73), direct degrada-

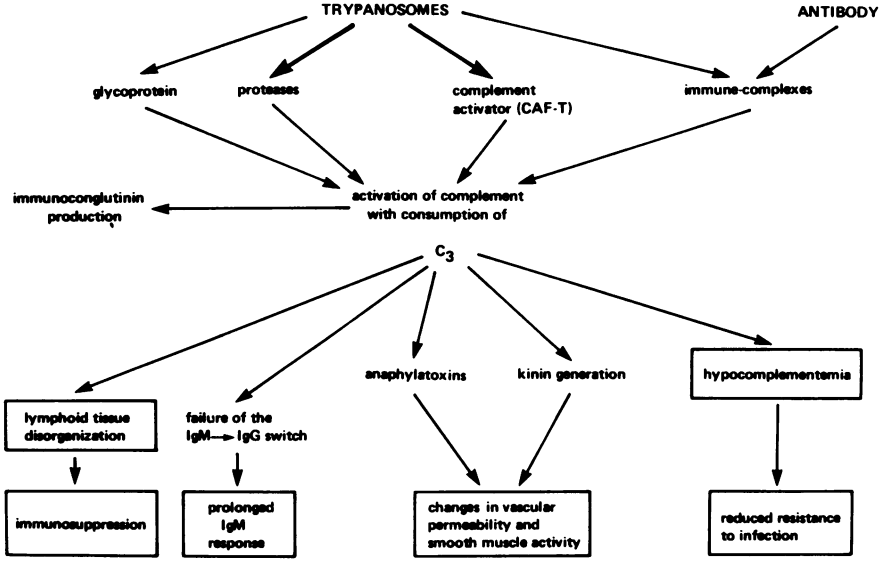

FIG. 3. *Possible mechanisms and consequences of hypocomplementemia in trypanosomiasis.*

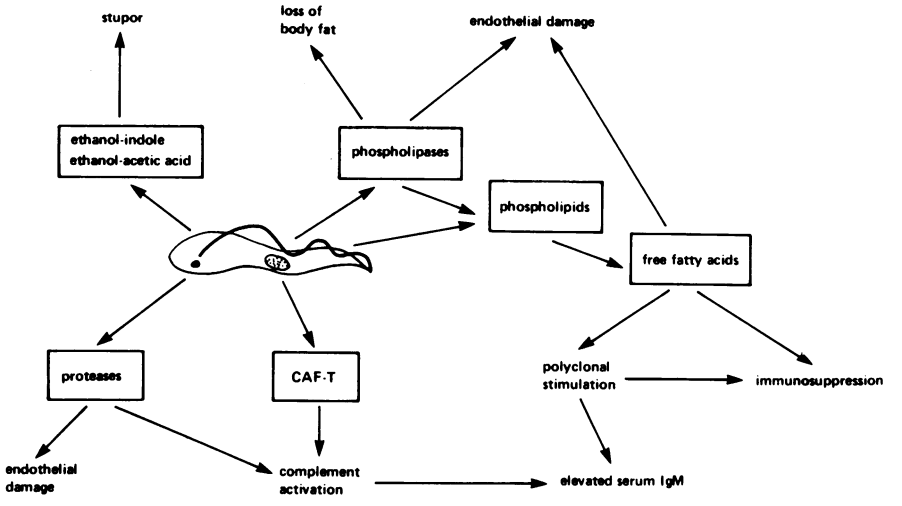

FIG. 4. *Major pathological activities of biologically active factors released from African trypanosomes.*

tion by phospholipases, and probably hemolysis or opsonization of erythrocytes mediated by cell-bound, complement-activating factors.

The occurrence of classical sleeping sickness, i.e., signs of severe neurological dysfunction and somnolence, are generally restricted to chronic infections of humans by *T. brucei gambiense*. The identification of potent anesthetics derived through tryptophan metabolism by this organism is obviously compatible with this. However confirmation of the significance of these compounds must await quantitative studies.

Although successful studies on the biologically active factors released by living, dead, or dying trypanosomes are relatively recent, it is becoming clear that these may account for much of the pathology of these diseases (Fig. 4). Whether or not these factors should be termed toxins is debatable. There are certainly no trypanosome toxins comparable to the secreted exotoxins of the clostridia or corynebacteria. Nevertheless, many may loosely be considered as endotoxins, i.e., toxic material released on autolysis. There is, indeed, no equivalent in the African trypanosomes to bacterial lipopolysaccharide. However, the phospholipases, fatty acids, lipopolysaccharides, and proteins do influence a wide spectrum of biological functions. Bearing in mind that trypanosomiasis is associated with prolonged periods of trypanolysis, it is not implausible that it is primarily through these factors that trypanosomes cause disease. It should also be borne in mind that the nonpathogenic trypanosomes, although they possess mitogens and are immunosuppressive (Hazlett and Tizard, Clin. Exp. Immunol., in press), do not show this persistent parasitemia. It is perhaps the prolonged destruction of trypanosomes in large numbers which enables the weakly toxic materials described here to cause sufficient disruption of cellular function that clinical disease and death may result.

CONCLUDING REMARKS

There is no doubt that trypanosomes contain or can release potentially pathogenic factors. However, the overall significance of these factors has not yet been demonstrated. Given the wide variety of lesions observed in the trypanosomiases, it is perhaps not surprising that causal connections may be devised between factors and lesions. Making these connections can, however, only be justified insofar as they enable hypotheses to be established. In this review we have presented the general hypothesis that at least some of the lesions observed in the African trypanosomiases are due to the actions of a number of trypanosome-derived factors. This suggestion must, however, remain hypothetical until more solid information, especially of a quantitative nature, becomes available. Given this additional information, we would anticipate that not all the processes outlined here will be confirmed and that, in general, the number of alternatives listed will be greatly restricted. Nevertheless, the accumulated data point to a significant role for trypanosome toxins in the pathogeneses of these diseases, a role which, in clarifying pathogenetic mechanisms, may provide us with a base on which to devise rational therapy for both humans and domestic animals.

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