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Trypanosomes

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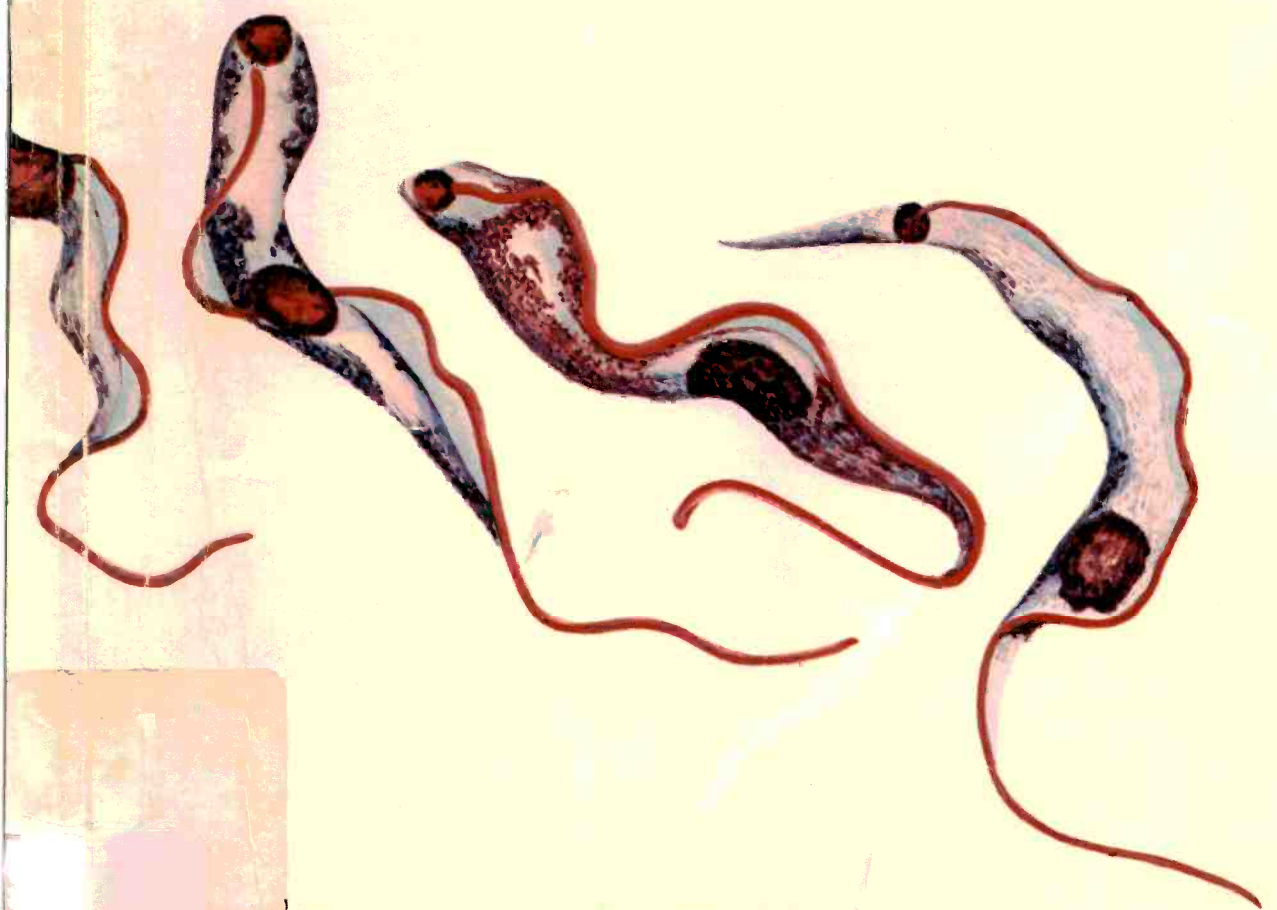
Proceedings of a workshop held at Nairobi, Kenya, 20-23 November 1978



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/IDRC publication/. Compilation of workshop papers on /trypanosomiasis/ particularly in /Africa south of Sahara/ - discusses the /metabolism/ of the trypanosome /parasite/s, mechanisms of /disease transmission/, effects on /blood/ and /serum/ /protein/ levels in /cattle/, /immunology/cal aspects, /disease resistance/.

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The metabolism of African trypanosomes in relation to pathogenic mechanisms

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Abstract. The metabolism of a parasite might affect its host in two ways: by depleting essential nutrients and/or producing toxic metabolites. The possibility that glucose consumption, pyruvate production, and the deamination of the amino acids tyrosine and tryptophan might be important in the effects of African trypanosomiasis is examined in the light of recent work.

In a recent discussion on the mechanisms of pathogenesis in African trypanosomiasis, Goodwin (1974) stated: "The pathogenesis is complex and the cause of death is still somewhat obscure. Damage to the tissues is brought about perhaps through the metabolic activities of the trypanosomes, more certainly through the repeated insults offered by the emergence of successive trypanosome variants and the attempts made to suppress them by the hosts' defence mechanisms." Our discussions at this conference will centre largely upon the nature of these "insults" and with the way the host responds to them, but it is my task in the next 30 minutes to consider the "perhaps" clause of Goodwin's comment. Thirty minutes is of course an impossibly short time in which to review present knowledge of trypanosome metabolism — a recent review of oxidative metabolism (Bowman and Flynn 1976) ran to more than 30 pages; on the other hand, it is a dauntingly long time in which to discuss the metabolism of these parasites in relation to their pathogenicity where, all too soon, one enters the realm of speculation. Thus, of necessity, this paper will be something of a compromise between the two. Clearly, the metabolic activity of a parasite might affect its host in two ways: by depleting essential nutrients and/or producing toxic metabolites. These two possibilities have been discussed in relation to trypanosomiasis for more than half a century, but we still lack firm evidence that either plays a key role in pathogenicity and in recent years attention has focused more on immunologic reactions and the release (from host tissues or damaged parasites) of pharmacologically active substances than on the effects of trypanosome metabolism. However, I am sure it is premature to dismiss the metabolic activity of trypanosomes as unimportant in pathogenesis — we still know far too little about the subject to do that.

Studies on the physiology and biochemistry of trypanosomes have tended to centre on carbohydrate metabolism and, in particular, on the changes in oxidative pathways associated with the developmental cycle of the *T. brucei* group (Newton, Cross, and Baker 1973; Vickerman and Preston 1976). The story that emerged is too well known to warrant detailed discussion here: the difference in cyanide sensitivity between blood and culture forms, the development of a functional cytochrome system in the latter, the differences in the end products of glucose metabolism, and the presence of the glycerophosphate oxidase pathway in blood forms are all well documented and have been the subject of several excellent reviews (see for example Fulton 1969; von Brand 1973; Bowman and Flynn 1976). In terms of pathogenic mechanisms, these aspects of metabolism seem singularly unpromising because, to quote Goodwin (1974) once more, "...none of the metabolites along the several pathways available are recognizable as dangerous poisons." However, one of the earliest, and for 30 years one of the most controversial, hypotheses on the cause of death in trypanosomiasis stemmed from the observation that the motility of trypanosomes in blood depends upon

adequate supplies of glucose (Schern 1928). It was suggested that so much glucose was consumed that the hosts' carbohydrate reserves became exhausted resulting in a breakdown of liver function and the onset of lethal hypoglycemia. This view was accepted by many workers, and evidence in support of it was published as recently as 1956 (reviewed by von Brand 1973); however, as von Brand points out, this hypothesis is not easily reconciled with a number of observations:

- Blood sugar levels return to normal, even in fasting animals, after administration of trypanocidal drugs (Scheff 1932).
- Liver glycogen reserves, although lowered, are not eliminated in infected animals (Mercado and von Brand 1960; Marciacq and Seed 1970; Lumsden, Merciacq, and Seed 1972; Ashman and Seed 1973).
- Feeding glucose to infected animals may prolong their lives for short periods but does not prevent the onset of terminal hypoglycemia (Andrews, Johnson, and Dormal 1930; Hoppe and Chapman 1947).
- The major end-products of glucose breakdown by trypanosomes are pyruvate and glycerol, which are readily metabolized by the host to produce a considerable amount of energy.

In the light of these and other facts, von Brand (1973) suggested that terminal hypoglycemia in trypanosomiasis is more likely to be due to a breakdown in hepatic or endocrine mechanisms controlling the mobilization of carbohydrate reserves than a direct result of the massive consumption of glucose by trypanosomes. However, it may be premature to dismiss this metabolic activity of the parasites as being of no consequence to the host: Voorheis (1969) has suggested that the continual demand for glucose by the parasite in acute infections may result in decreased glucose metabolism in the hosts' peripheral tissues leading to a condition resembling diabetes mellitus. This idea merits further investigation.

Another aspect of the high glucose consumption by bloodstream trypanosomes is the production of pyruvate and how it relates to pathogenesis. Although pyruvate is readily used by host tissues, it has been shown in laboratory infections to accumulate in the blood in amounts directly proportional to the numbers of parasites present (Grant and Fulton 1957; Coleman and von Brand 1957). High concentrations of pyruvate could lead to depletion of alkali reserves, acidosis, and a lowered affinity of hemoglobin for oxygen. Naus and Yorke (1911) drew attention to the dark purple

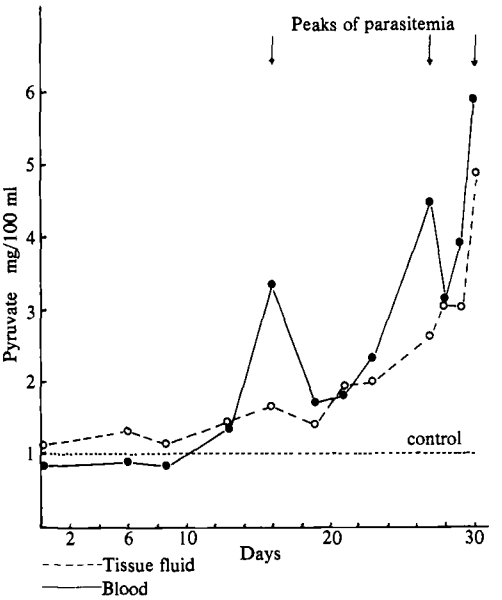

Fig. 1. *Effect of* T. brucei *infection on blood and tissue fluid pyruvate levels in rabbit (from Goodwin 1974).*

colour of blood in late stages of *T. brucei* infections, and Andrews, Johnson, and Dormal (1930) proposed that deficient oxygenation of hemoglobin coupled with mechanical blockage of the circulation leads to death by asphyxiation. Coleman and von Brand (1957), on the other hand, conclude that pyruvate does not reach a generally toxic level in the bloodstream. It must be stressed, however, that the *T. brucei* group, which are humoral rather than hematic parasites (Losos and Ikede 1972), may very well produce toxic levels of pyruvate at extravascular sites. Goodwin and Guy (1973) using their elegant "hair-curler technique" have studied pyruvate levels in blood and tissue fluids of *T. brucei*-infected rabbits and have found increases to five times the normal value (Fig. 1). They suggest that the high concentrations of pyruvate in tissue fluid may be associated with the observed changes in the structure of connective tissues covering the subcutaneously implanted hair curlers. In control animals this connective tissue, which is composed mainly of collagen fibres, fibroblasts, and blood vessels, is smooth, well organized and free from lipid, whereas in *T. brucei*-infected animals it has a rough surface. Moreover, the fibroblasts in infected animals contain lipid droplets, cease to produce collagen fibres, and may become detached into the surrounding tissue fluid (Goodwin, Guy, and Brooker 1973). Discussing these results, Goodwin (1974)

points out that although fibroblasts normally produce and store lipids, they rarely exhibit large lipid droplets in cytoplasm (Noble and Boucek 1955). Fibroblasts cultured in the presence of excess fatty acids do accumulate such droplets. Thus, it seems possible that the high in vivo concentrations of pyruvate, which occur in *T. brucei* infections, produce a similar effect on fibroblasts and are responsible for, or at least contribute to, the degenerative changes observed in the connective tissues of infected rabbits. In considering this possibility, it is interesting to note that infections with hematic trypanosomes (*T. congolense* and *T. vivax*) are not characterized by extensive inflammatory, degenerative, and necrotic changes and that these organisms do not produce as much pyruvate per mole of glucose metabolized as do *T. brucei* group trypanosomes (Fig. 2).

Compared with our knowledge of carbohydrate metabolism, we know relatively little about the metabolism of amino acids by hematozoic trypomastigotes. Tracer experiments have shown that alanine is the major amino acid produced from glucose by *T. rhodesiense* and *T. gambiense* (Grant and Fulton 1954; Shaw, Voller, and Bryant 1964; Chappell, Southworth, and Read 1972). A number of other amino acids are also labeled (aspartate, glutamate, glycine, and serine), and there is evidence that trypanosomes can interconvert some of them, but there is no estimate of the proportion of the total amino acid requirement for growth that is satisfied by *de novo* synthesis. Exogenous amino acids are known to enter trypanosomes by both diffusion and specific transport systems (Voorheis 1973; Jackson and Fisher 1977), and there is evidence that blood forms of *T. brucei* are capable of ingesting and digesting proteins (Langreth and Balber 1975). Again, the relative importance of these processes in satisfying the organism's requirements is unknown. It has been suggested (de Raadt and Seed 1977) that the availability of ingestible proteins, amino acids, and other nutrients is an important factor in determining when, during an infection, trypanosomes begin to develop in the cerebrospinal fluid (CSF). The parasites may have access to the CSF throughout an infection (Peruzzi 1928b) but be unable to grow until it has been enriched by proteins and other nutrients from degenerating tissues.

How much stress to the host's nitrogen metabolism results from the parasites' demand for amino acids is unknown. Serum and tissue fluid albumin levels fall steadily during the course of a *T. brucei* infection in rabbits, and levels of nonprotein nitrogen (particularly proline, alanine, creatinine, and urea) rise (Goodwin and Guy 1973). A detailed

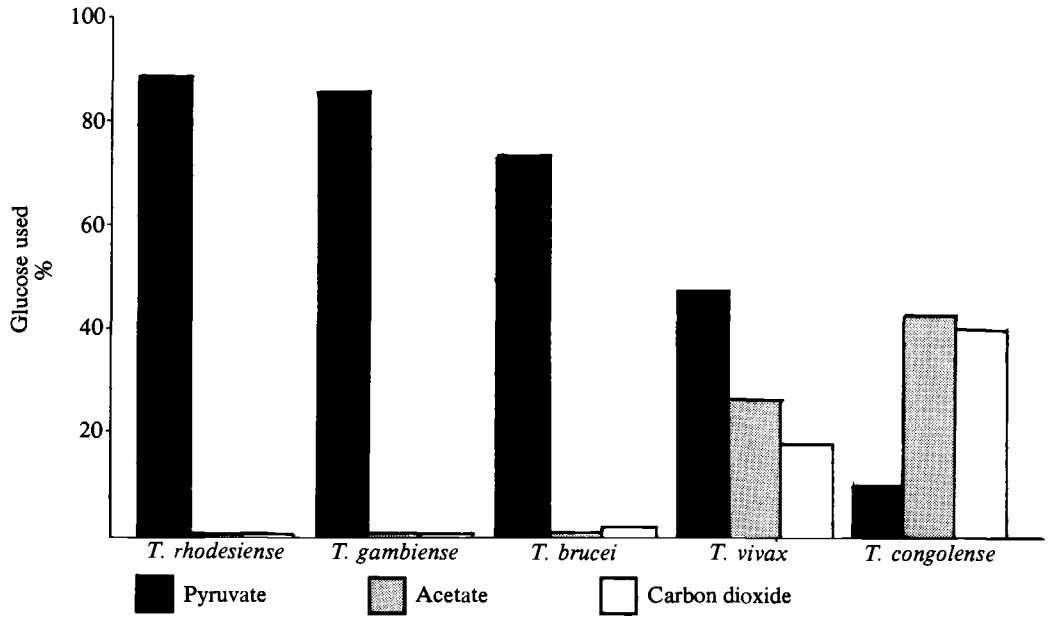

Fig. 2. *Pyruvate, acetate, and carbon dioxide resulting from the metabolism of glucose by blood forms of hematic and humoral trypanosomes (from Ryley 1956).*

Table 1. Serum and hepatic tyrosine aminotransferase activity in uninfected and *T. gambiense*-infected voles.

Group	Serum (nmoles substrate/mg protein/h)	Hepatic (μmoles substrate/mg protein/h)
Control	0	2.29
T. gambiense-infected	17.66	5.43

Source: Stibbs and Seed (1976).

study of free serum amino acids in voles (*Microtus montanus*) infected with *T. gambiense* has also revealed major changes (Newport et al. 1977). In control animals, the majority of amino acids showed diurnal variation, levels being highest during the dark period; this pattern was not found in infected animals and the levels of 7 (threonine, serine, valine, isoleucine, leucine, tyrosine, and tryptophan) of 18 amino acids studied fell significantly below the levels in the controls. In agreement with Goodwin and Guy's (1973) rabbit experiments, it was found that alanine and proline levels were markedly increased at certain stages of the infection. Of the amino acids that were reduced, tyrosine and tryptophan were most affected: tyrosine to about 50% of control levels and tryptophan to undetectable levels. The fall in tyrosine was predicted by Stibbs and Seed (1976) when they found elevated serum and hepatic tyrosine aminotransferase levels in *T. gambiense*-infected voles (Table 1). They point out that tyrosine metabolism has seldom been investigated during a parasitic infection, although this amino acid is an important precursor of catecholamines (Fig. 3). Goodwin (1970) drew attention to the fact that norepinephrine alleviates the shock that accompanies protozoal infections and suggested that catecholamine metabolism is defective in African trypanosomiasis; the work of Stibbs and Seed (1976) and Newport et al. (1977) seems to support this idea given that in other mammalian systems a fall in serum tyrosine, relative to other neutral amino acids, restricts tyrosine transport across the blood-brain barrier and lowers derivative catecholamine pools in the brain (Wurtman et al. 1974). In keeping with these findings, Newport and Page have found a reduction of 32–45% in brain, liver, and skeletal muscle tyrosine in *T. gambiense*-infected voles (Table 2). Speculating on the significance of these results, Stibbs and Seed (1976) suggest that a reduced brain tyrosine level accounts for some of the neurological syndromes occurring in *T. gambiense* infections; it is known that depression of catecholamine biosynthesis results in changes in sleep or activity patterns (Jouvet 1969), body temperature (Svensson 1971), glycogen, and lipid metabolism and possibly causes mental depression (Schildkraut 1965). Similarly, it is possible that the observed fall in serum tryptophan (Newport et al. 1977) could result in decreased synthesis of niacin and serotonin by the host, leading to a pellagra-like syndrome, changes in sleep patterns, and depression (Stibbs and Seed 1975a). At present, we cannot judge just how much of the reduction in serum tyrosine and tryptophan is

Table 2. Tyrosine levels in brain, liver, and muscle tissue from uninfected and *T. gambiense*-infected voles.

Tissue	Control	*T. gambiense*-infected
Brain	0.107	0.059
Liver	0.265	0.181
Muscle	0.187	0.103

Source: Newport and Page (1977).

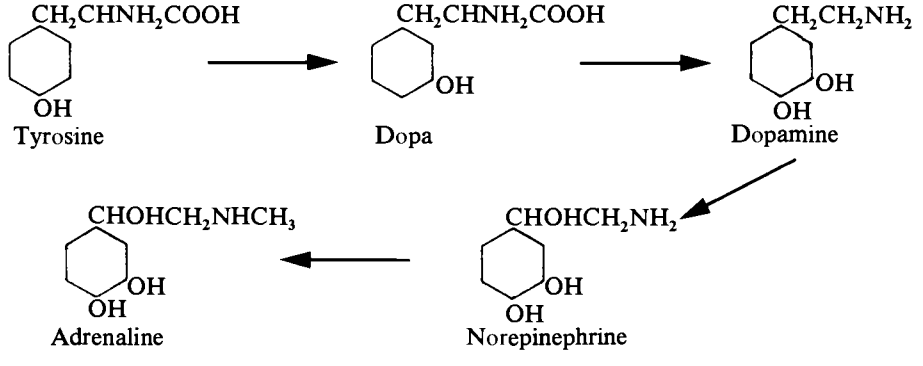

***Fig. 3**, Metabolic pathway for catecholamine biosynthesis from tyrosine.*

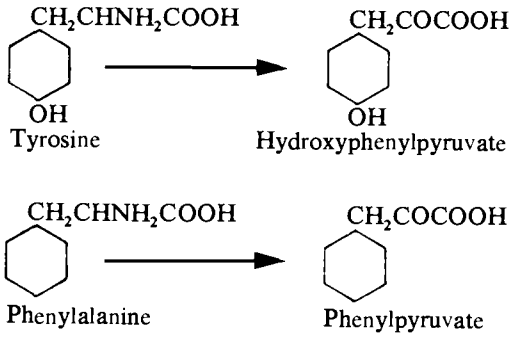

Fig. 4. *Deamination products of tyrosine and phenylalanine.*

due to the metabolic activity of viable trypanosomes, changes in host metabolism, or the release of enzymes from degenerating cells; the work of Stibbs and Seed (1976) suggests that tyrosine aminotransferase is released from trypanosomes after agglutination and lysis by variant-specific antibody. We do know that both tyrosine and tryptophan are actively metabolized by *T. gambiense*, so it seems reasonable to discuss these effects under the title of this lecture.

There is increasing evidence that the accumulation of end-products of amino acid metabolism contributes to the characteristic pathology of African trypanosomiasis. Transamination of tyrosine yields p-hydroxyphenylpyruvate (Fig. 4), high concentrations of which have been found in the urine but not in the blood of infected animals (Stibbs and Seed 1975c, 1976). This metabolite is closely related to phenylpyruvate (formed by transamination of phenylalanine), which is a known inhibitor of adrenaline synthesis. Accumulation of either of these deamination products in the brain could contribute to the pathological picture of Gambian sleeping sickness. Clearly, this is an area demanding further research. Similarly, deamination of tryptophan yields pharmacologically active compounds, and considerable interest in these has been aroused by the work of Seed's group. Indole pyruvate, the immediate product of tryptophan transamination, is further metabolized to indole lactate, indole acetate, and indole ethanol (tryptophol) (Fig. 5) (Stibbs and Seed 1973, 1975a, b, and d). There is evidence that tryptophol can cause sleep, convulsions, and death by respiratory depression when injected into mice, rats, and cats (Sabella et al. 1969), and it has been suggested that trypanosomes in extravascular sites of the central nervous system produce sufficient quantities of this indole to produce similar effects. Tryptophol has also been reported to cause immunodepression in laboratory rodents (Ackerman and Seed 1976) and may contribute to it in humans and other animals. It is thought that tryptophol acts on cell membranes, perhaps by combining with the outer lipid bilayer (Tizard et al. 1979), and in support of this, recent

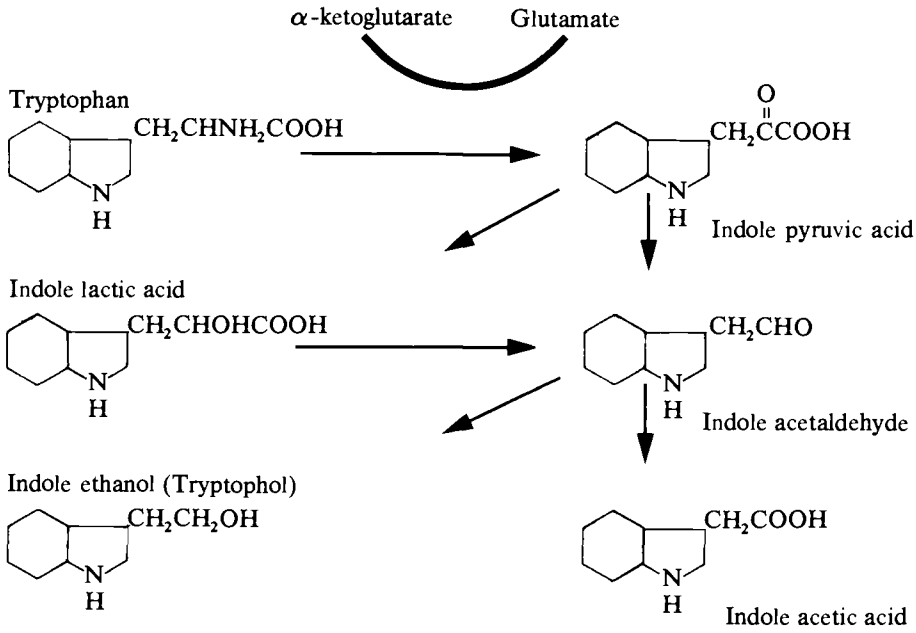

Fig. 5. *End products of tryptophan metabolism by* T. gambiense *(from Stibbs and Seed 1975a).*

work (Seed, Seed, and Sechelski 1978) has shown that tryptophol rapidly lyses red blood cells. Similar action on synaptic membranes may cause changes in the transmission of nerve impulses, give rise to behavioural changes, and induce a sleep-like or comatose state.

An essential step in testing the hypothesis that tryptophol produced by trypanosomes is important is the determination of in vivo levels of the metabolite during infection and the correlation of these with the various states observed. This work is in progress in Seed's laboratory. As a first step, Seed and Hall (1977) have estimated from in vitro measurements that a minimum of 3.2 mg tryptophol/kg body weight could be formed in an infected mouse. This level is, they believe, compatible with their hypothesis, which is further supported by the finding that levels of indole lactate and indole acetate in the urine of trypanosome-infected mice are about three times those found in control animals.

It is not yet possible to say from any of this work that tryptophol or other metabolites of aromatic amino acids are responsible for the abnormal behavioural and/or pathological changes observed in infected animals, but the investigations by Seed and his collaborators have opened up a whole new area of research that may lead to an explanation of at least one aspect of African trypanosomiasis. Mental disturbances are often found (80–95%) in chronic human infections (Lambo 1966); histological studies have thrown no light on their cause, and the behavioural syndromes of the disease remain a clinical mystery. Perhaps the answer will come from detailed studies of trypanosome metabolism and investigation of the pharmacological activity of the metabolites they produce.

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