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Pathogen Transmission in Relation to Feeding and Digestion by Haematophagous Arthropods

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Introduction

The blood feeding habit, especially among opportunist feeders such as tabanids and *Stomoxys* is known to result in transmission of diseases for which the vectors are not the obligate or alternate hosts. Thus, mechanical transmission of trypanosomes such as *T. vivax* can occur in cattle herds outside tsetse fly areas where tabanids are actively feeding. In the case of Yaws, mechanical transmission of the spirochaetes by eye flies *(Hippelates pallipes)* in the West Indies is thought to be most likely. The spirochaetes remain motile in the pharynx and oesophageal diverticula for several hours but are apparently immobilised in the midgut (KUMM & TURNER, 1936). There is apparently no development of spirochaetes in the fly. They have been shown to pass through houseflies, but in mechanical transmission, biochemical transformation or adaptation of the pathogen is not implied.

Virus transmission is common among arthropods and transovarial transmission to succeeding generations is frequent in mites and ticks. Although Yellow Fever virus is not transovarially transmitted by its vector *Aedes aegypti,* the mosquito only becomes infective some time after ingesting an infected blood meal (CHANDLER, 1955). Thus, metabolic or biochemical changes or adaptations in the virus or in the vector are in some way implicated, as they must also be in transovarially transmitted viruses. However, the causal relationships between virus infectivity and vector physiology are poorly understood.

As with virus infections, those disease organisms possessing a cyclical host/vector relationship will possess a variable infectivity potential which is not necessarily related to the abundance of any of the organisms in the cycle. Clearly, feeding behaviour and host preferences of the vector are important in determining the rate and extent of disease transmission, and such parameters can be quantified in epidemiological studies. However, a complete understanding of the factors concerned in cyclical disease transmission also depends on a knowledge of the physiology of the organisms involved, and particularly of the interdependence of their physiologies. The subject is vast, and it is proposed to illustrate the problems involved and the progress made, by reference largely to trypanosome transmission by tsetse flies (*Glossina* spp.).

Developmental adaptations to the invertebrate host

Although only a few tsetse fly species are implicated as vectors of trypanosomiasis in Africa there is no evidence that any species is incapable of cyclically transmitting trypanosomes. Of the polymorphic trypanosomes which invade the salivary glands of *Glossina, T. brucei*

gambiense does not easily infect laboratory animals while being infective to man. *T. b. rhodesiense* is virulent to man and to laboratory animals, but *T. b. brucei* is highly virulent to laboratory animals while man is not susceptible. Thus, much of our knowledge of the polymorphic trypanosomes has been gained from *T. b. brucei.* The monomorphic *T. vivax* develops in the tsetse proboscis and *T. congolense* is found in the intestine and mouthparts of *Glossina.*

In the mammalian blood stream two morphologically distinct forms of *T. b. brucei* are recognisable, one long and slender, and the other short and stumpy. VICKERMAN (1971) has shown that the morphological difference is associated with differences in the respiratory metabolism of the trypanosome; the slender forms are wholly glycolytic whereas the stumpy forms show evidence of mitochondrial oxidative decarboxylation and have greatly increased mitochondrial volume. Since the mitochondrion is the principle energy provider for the trypanosome in the tsetse midgut, the stumpy forms may be regarded as pre-adapted to infection of the vector. Neither *T. vivax* nor *T. congolense* are polymorphic (pleomorphic), nor do they show mitochondrial changes. However, their mitochondria more closely resemble those of the stumpy forms of *T. b. brucei,* and additional oxidases such as succinoxidase (developed only in procyclic *T. b. brucei*) are found in these monomorphic trypanosomes. The trigger for conversion of *T. b. brucei* procyclic forms is unknown but may perhaps be associated with reduced carbohydrate availability in the vertebrate host blood, and the kinetoplast (mitochondrial DNA) is thought to be involved.

Recently HARMSEN (1973) has noted further biochemical changes in *T. b. brucei* following ingestion by *G. pallidipes* which could be associated with transformations necessary for the trypanosome to infect the fly. Briefly, the trypanosome leaves the homeostatic environment of the vertebrate blood stream at 37 °C and is cooled to 20–25 °C upon entering the crop of the fly. If the fly is less than 24 h old its peritrophic membrane, which is sac-like, is not large and must grow to accommodate the blood meal. Thus the trypanosome may remain for between 1 and 3 h in the crop of the fly before being transferred to the midgut. During this period in the crop an enzyme transformation (glucose-6-phosphatase) takes place in the trypanosome. If the fly is older than 24 h when it takes its infected blood meal the peritrophic membrane has already grown large enough to accommodate the blood meal, which is transferred from the crop to the midgut within one hour of feeding. This period is too short for the enzyme transformation to have occurred in the trypanosome before it is faced with the potentially hostile environment of the midgut. This reasoning is advanced by HARMSEN to explain the loss of infectivity of tsetse flies which take infected blood meals when they are more than 24 h old. However, the potential hostility of the

midgut environment is difficult to define. Although water is extracted from the blood meal very rapidly after transfer to the midgut (BURSELL, 1960), proteolytic enzymes are confined to the posterior portion of the midgut and maximum enzyme activity is not achieved until 20 h after ingestion of a blood meal (LANGLEY, 1967). In mosquitoes, blood meals are passed directly to the midgut or stomach without entering the diverticulum. The mosquito peritrophic membrane is formed by droplet secretion from cells of the midgut and condenses around the blood meal. It is here that gametocytes of *Plasmodium* spp. ingested by the mosquito form male and female gametes, and that zygote formation occurs. After some development the ookinete penetrates the gut wall of the mosquito to continue its development in the environment of the haemocoel, resulting sporozoites finally reaching the salivary glands. The proteolytic enzymes involved in blood digestion do not reach maximum activity until about 18 h after feeding (FISK & SHAMBAUGH, 1952) and recent work suggests that survival of gametocytes, gametes and young zygotes in the midgut is made possible by this delay in appearance of active enzyme (T. Freyvogel – personal communication). A temperature dependent transformation in *Plasmodium* may also be demonstrated in that microgamete formation by exflagellation can occur *in vitro* simply by cooling from the mammalian host temperature.

There are clearly analogies here between aspects of development in *Trypanosoma* spp. and *Plasmodium* spp., but the manner in which micro-organisms, pathogens or symbionts, adapt to the seemingly hostile environment of the host alimentary tract is not understood. It seems, however, that opportunities do exist for sensitive stages to complete their development before the onset of hostile conditions. The possible role of invertebrate trypsin inhibitors in vertebrate serum (GOODING, 1972, 1974) affording initial protection from the host enzymes cannot be ignored in this context.

It is generally agreed that if *T. b. brucei* pass through the proventricular valve of a tsetse fly and enter the midgut in the correct physiological state, they next appear in the ectoperitrophic space close to the proventriculus. There is, however, no general agreement as to how they arrive there. They may either pass along the digestive portion of the midgut to escape from the peritrophic membrane at its distal end, or they may pass through a rupture in the peritrophic membrane, or again they may pass directly through the membrane at its proximal point of secretion close to the proventriculus (FREEMAN, 1973). By whatever route they reach the proventricular ectoperitrophic space, their arrival there seems to be a pre-requisite for subsequent development to metacyclic forms in the salivary glands which are capable of re-infecting vertebrates. During their active proliferation phase in the tsetse gut the main energy source for the trypanosomes is proline

(SRIVASTAVA & BOWMAN, 1971) which is probably available from the digested blood meal that originally contained them.

The migration of *T. b. brucei* proventricular forms from the ectoperitrophic space to the salivary glands is thought to involve a return to the endoperitrophic space (FAIRBAIRN, 1958) whence they pass up the oesophageal lumen and enter the salivary gland ducts. However, MSHELBWALA (1972) has shown that all forms of *T. b. brucei* typical of the development cycle in tsetse can be found in the haemocoel of at least three species of *Glossina*. Non-metacyclic forms were not infective to mice but metacyclic forms found in the haemolymph produced infections following inoculation into mice. Although no positive evidence that these haemolymph trypanosomes enter the salivary glands of the fly has yet been obtained, the observations clearly indicate an alternative developmental pathway to that previously indicated.

The role of culture techniques

A major requirement for the understanding and interpretation of physiological and biochemical relationships between vector and pathogen is the development of laboratory culture techniques for the organisms concerned. The necessity of providing small laboratory mammals for biochemical studies, whose genetic and nutritional homogeneity can be guaranteed and defined has long been appreciated and commercially exploited, but only recently has progress along similar lines been made with invertebrates.

Culture of trypanosomes

Laboratory culture of trypanosomes is achieved by syringe passage in small laboratory animals, serial sub-culture in various nutrient media, or by preservation as stabilates at low temperature. Cultivation *in vitro* is difficult and according to NEWTON et al. (1973) only those forms which correspond to the procyclic invertebrate trypomastigotes can be maintained indefinitely in culture. Even so, culture media have been complex and undefined since they all contain vertebrate blood components (TAYLOR & BAKER, 1968). Recently, however, CROSS and MANNING (1973) have developed a defined medium which supports the growth of culture forms of *T. b. brucei* and *T. b. rhodesiense*. The authors point out that their medium does not provide the minimal requirements for trypanosome growth and that they have not yet explored the area of trace element requirements. Nevertheless, they believe that their approach provides a possible solution to the problem of cultivation of vertebrate blood stream forms, and further developments should render biochemical and physiological investigations more meaningful.

The development of continuous flow techniques for the culture of blood protozoa *in vitro,* in which waste products are removed from the medium by dialysis (COWPER et al., 1972) may also result in improved and defined environments for the study of their physiology.

Another approach to *in vitro* trypanosome culture has involved media containing certain tsetse fly tissues (TRAGER, 1959; CUNNINGHAM, 1973). The latter author found that *T. b. brucei* multiplied best in a medium containing whole alimentary tracts. Similar growth was observed when tsetse guts were isolated from the trypanosomes in the medium by a permeable membrane. No growth was obtained in (a) culture medium alone, (b) media containing extracts of the alimentary canal, or (c) media in which alimentary tracts had been cultured for several days. This implies that the continued growth of trypanosomes requires a supply of diffusable substances from the living alimentary tract.

Culture of blood stream forms of trypanosomes *in vitro* has shown that haematin is essential to growth, but in addition whole living erythrocytes are apparently necessary. It seems that the trypanosomes utilise the glucose-6-phosphate dehydrogenase (G6PD) system of the red cell, and it has been suggested that trypanosome specificity to certain hosts may be associated with G6PD differences between vertebrates of different species (FROMENTIN, 1972).

Culture of *Glossina* spp.

Apart from the pioneer experiments of GEIGY (1948) with *Glossina palpalis* fed on guinea pigs, successful culture of *Glossina* spp. using living vertebrate host animals has only been achieved during the last ten to fifteen years (see NASH et al., 1971), but many other haematophagous arthropods have been cultured in a similar way for much longer. The unusual mode of reproduction in tsetse flies (adenotrophic viviparity) coupled with the obligatory haematophagous habit posed special problems, but apart from a slower post-eclosion development of the adult flight musculature (BURSELL, 1961; LANGLEY, 1970) attributable to reduced flight activity in captivity (BURSELL & KUWENGWA, 1972) at least one tsetse species in laboratory culture *(G. morsitans)* appears to have retained its normal behavioural and physiological potential as evidenced by normal rates of dispersal and survival when released under field conditions to compete with natural populations (DAME & SCHMIDT, 1970).

Serious attempts to rear tsetse flies on vertebrate blood presented in an *in vitro* feeding system began with the description of a suitable membrane through which the flies would willingly feed (LANGLEY & MALY, 1969). Varying degrees of success have been re-

ported (LANGLEY, 1972; MEWS et al., 1973; LANGLEY & PIMLEY, 1975) but recently *G. morsitans* has been maintained for several generations with no apparent deleterious effect upon reproductive performance, by feeding the flies exclusively upon fresh defibrinated pig blood (MEWS et al., 1975). This must be regarded as a significant step towards the development of a defined diet for *Glossina* spp. and is believed to be the first occasion upon which a haematophagous arthropod that relies on vertebrate blood as a nutrient source for the whole of its life cycle has been reared successfully for more than a few generations in the absence of a living host animal.

Thus, progress is being made in defining the nutritional requirements of the African trypanosomes and their tsetse fly vectors. The combined use of *in vitro* culture techniques for both organisms would seem to present a hopeful approach to the better understanding of factors involved in cyclical transmission of trypanosomiasis. There seems every reason to suppose that similar approaches with other haematophagous arthropods and their associated pathogens should increase our knowledge considerably, with obvious implications for the control of disease.

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