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PREFACE

This volume of Advances in Parasitology continues the policy established by Ben Dawes and developed by Professor Lumsden, of attempting to review any aspect of parasitology in which significant developments are being, or have recently been, made. We feel that the traditional division of parasitology into protozoology and helminthology is becoming increasingly artificial. As more emphasis is laid on the subject's ecological aspects—including interrelationships between parasites and their hosts—and on the cell biology of the parasites themselves, the common principles resulting from a shared life-style and a common eukaryotic nature, are becoming more evident. We hope to include in future volumes, papers dealing with general principles of parasitism, not subdivided on the basis of uni- or multicellularity and not necessarily restricted even to eukaryotic organisms.

Meanwhile, in the present volume, the traditional division is maintained, though our first criterion—topicality and significance—is, we believe, fully met by all the included papers. Perhaps the most controversial contribution is that by Evans and Ellis, questioning views which have been held more-or-less uncritically since the work of Muriel Robertson early this century.

1983

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I. Introduction

Research work carried out over the past 10 to 15 years has given new insight into many of the problems associated with African trypanosomiasis. The discoveries in the laboratory, for example, of antigenic variation, of switches in metabolic pathways and of variations in the isoenzyme patterns of differing trypanosome populations have produced a clearer understanding of some of the complex interrelationships between hosts, parasites and vectors. In the field, however, many of the early problems remain.

As long ago as 1921 Duke noted that it was sometimes impossible to find trypanosomes in the salivary glands of tsetse flies caught in an area where transmission of trypanosomiasis was occurring. Buxton (1955) reviewed the problem, and the difficulty generally of correlating the extent of the disease found with the apparent degree of tsetse involvement. While Molyneux and his colleagues (1979) have shown how the behaviour of infected flies would tend to maximize transmission, there still may be other factors involved, and the behaviour of the trypanosomes described here may have some bearing on these and other problems long recognized in sleeping sickness and related trypanosomiases.

A. The traditional view of the developmental pathways of African trypanosomes within the tsetse fly

The parasites responsible for these diseases are those of the *Trypanosoma brucei* group; the generally accepted developmental pathways during that part of their life cycle which is within their vector, the tsetse fly (*Glossina* spp.), have been described by Buxton (1955, pp. 607–609) as follows.

> "If a tsetse takes blood containing trypanosomes (setting aside the possibility of direct transmission) the organisms may disappear, either while the meal of blood is being digested, or during the digestion of a subsequent meal: or they may survive in the crop, living there for many days but failing to establish themselves elsewhere (*T. rhodesiense* in *G. palpalis*, Duke and Mellanby, 1936); or they may establish themselves first in the midgut, later in the salivary glands and so complete the cyclical development. In the last case, the fly becomes capable of transmitting the infection to other mammals. The cycle is only completed in a very small proportion of flies, under normal circumstances (...).
>
> "In the event of the trypanosomes establishing themselves, they pass with the blood into the midgut, and at first lie inside the tubular peritrophic membrane which separates them from the epithelium of the midgut (...). After about 4 days they may be found in the ectoperitrophic space, i.e. outside the membrane, between it and the epithelium (Yorke,

Murgatroyd and Hawking, 1933). It appears highly probable that they have reached this position by passing down the alimentary canal inside the peritrophic membrane, as far as its free posterior end, which is in the hindgut; from that point they probably pass from within the membrane to the ectoperitrophic space, in which they migrate forward along the midgut to the proventriculus. . . .

"Having passed right forward in the ectoperitrophic space, the parasites find themselves in the annular space with midgut epithelium on one side and the base of the peritrophic membrane, in the proventriculus, on the other (...). It is probable that they escape by passing through the base of the peritrophic membrane at the point where it is being actively produced and is still fluid."

Thus, according to this pathway, the trypanosomes pass through the wall of the peritrophic membrane only to enter the endoperitrophic space, not to leave it. Having re-entered the endoperitrophic space, Buxton suggests, "the trypanosomes . . . might pass forward through the foregut and food canal to the distal extremity of the hypopharynx: from that position it is held that the organisms pass up inside the hypopharynx from tip to base, and so by way of the ducts to the salivary glands. It cannot be claimed that every stage of this cycle has been observed, indeed it would be a matter of extreme difficulty to obtain direct evidence of some parts of it."

Figure 1 shows the whole of this complex pathway diagrammatically. Figure 1(a) is usually stated to be based on the various observations and published work of Bruce and Robertson in the 20 years before the First World War. But their version, as will be seen from Fig. 1(b), did not involve the presence of the peritrophic membrane or a very clear journey to reach the open end of the hypopharynx that the parasites would have to travel to reach the salivary glands.

B. MAJOR CONTRIBUTIONS SUPPORTING THE TRADITIONAL VIEWS

It is necessary to examine here how the accepted developmental pathways (as quoted above, from Buxton, 1955) were built up, not only to establish how this was achieved historically, but also to relate the recent observations reported here to those of the original workers.

The first demonstration of the association between trypanosomes, thought to be *T. congolense* (Hoare, 1972), and tsetse flies (*G. morsitans*) was by Bruce (1895, 1897) who showed that tsetse could be used to transmit "live viruses" among horses. Later, Brault (1898) suggested that this could be the method of spread of human sleeping sickness. In 1903 Bruce and Nabarro reported from Uganda that wild-caught tsetse were able to transmit trypanosomiasis to monkeys. In the same year, from Zululand, Bruce noted that the

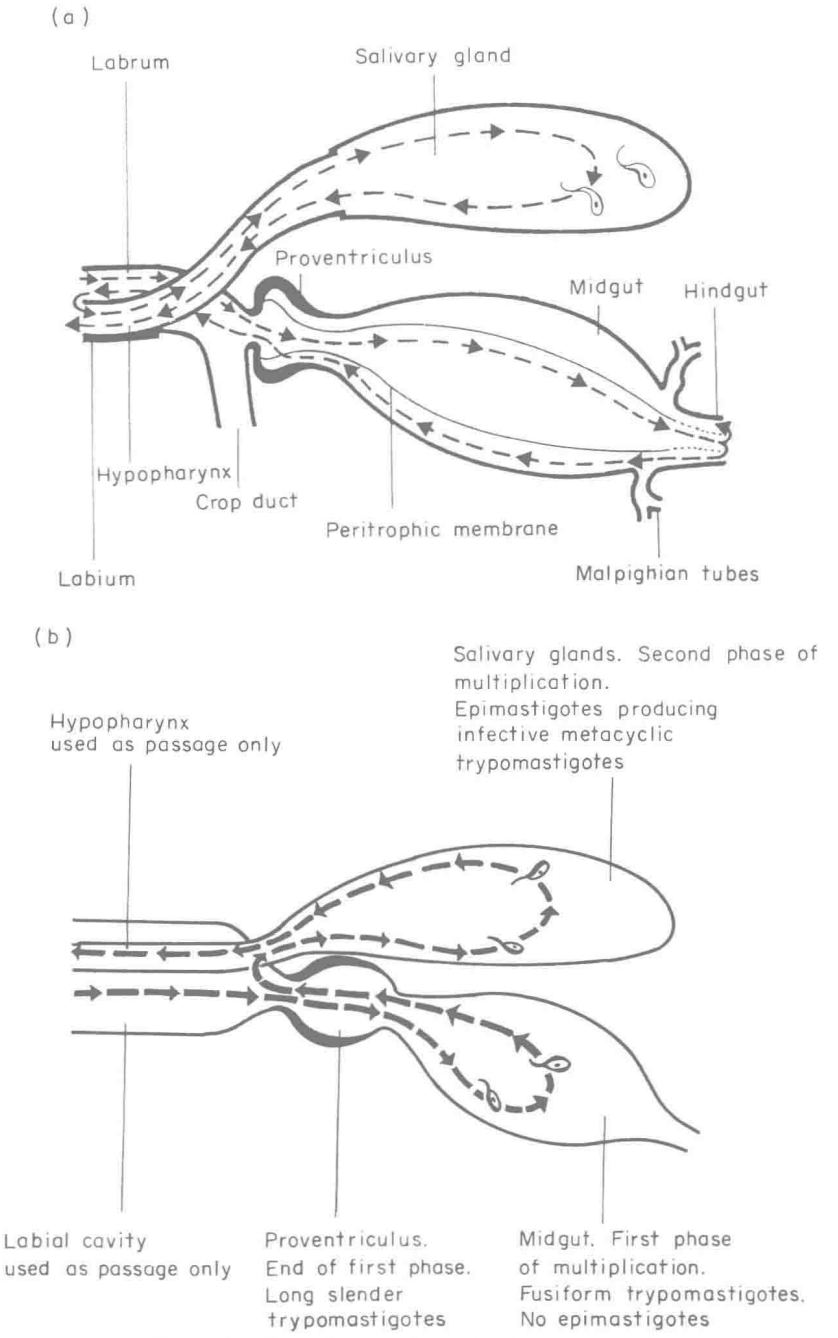

FIG. 1. Diagrams illustrating the pathway followed by *Trypanosoma brucei* within *Glossina* spp.: (a) as outlined by Buxton (1955; based on diagram by Davies, 1967); (b) adapted from diagram by Wenyon (1926).

trypanosomes found in tsetse guts were not infective to mammals (Bruce, 1903), yet the disease could be passed by flies (*G. palpalis*) from sleeping sickness patients to monkeys for up to 48 hours after the flies fed on these patients (Bruce *et al.*, 1903). Some form of development within the fly therefore seemed possible, and Kleine (1909) showed that a cycle did exist in his laboratory-reared *G. palpalis* flies, taking 20 days, this being the time between an infective feed and the moment when the flies first became capable of transmitting *T. brucei*. Bruce *et al.* (1909) reported that Kleine's tsetse remained infective for at least 50 days after infective feeds, and confirmed Kleine's findings using *T. gambiense*, then the only recognized cause of human sleeping sickness. They also reported completion of the cycle in tsetse salivary glands with the production of infective metacyclic forms. When the salivary glands were packed with trypanosomes, none was found in the "proboscis, proventriculus, thoracic gut, crop, hindgut and Malpighian tubules" but they were seen in the midgut from the fourteenth to the twenty-third day. Bruce noted also the low rate of fly transmission: 1 % of *G. morsitans* with *T. brucei* and 0.2 % of *T. gambiense* with *G. palpalis*. Two years later (Bruce *et al.*, 1911c), he had raised the percentage to 8 in some experiments with *T. gambiense*.

In 1911 Bruce *et al.* (1911a, b) summarized their findings as follows.

(a) *T. gambiense* multiplied in the gut of about one in every 20 *G. palpalis* which had fed on infected animals.
(b) Flies became infective, on average, 34 days after their first feed.
(c) Flies may remain infective for 75 days.

Later in the same report (Bruce *et al.*, 1911a) they added the following conclusions.

(a) In the course of development of *T. gambiense* in *G. palpalis* the proboscis did not become involved as in the case of some other species.
(b) A few days after the infective feed the trypanosomes disappeared from the great majority of the flies, but in a small percentage this initial disappearance was followed by renewed development.
(c) After a very short time the flies which had fed on an infective animal became incapable of conveying the infection by their bites, and this non-infectivity lasted for 28 days, when renewed or late infectivity developed.
(d) A fly in which this renewed or late infectivity occurred could remain infective for at least 96 days.
(e) Invasion of the salivary glands occurred at the same time as this renewal of infectivity, and without this invasion of the salivary glands there could be no infectivity.
(f) The type of trypanosome found in the salivary glands when the fly became infective was similar to the short stumpy form found in the

vertebrate's blood, and it was believed that this reversion to blood type was *sine qua non* in the infective process.

They also noted that, while other trypanosome forms were found in the salivary glands, the only place within the fly where the blood-like type was found was in these glands.

Throughout much of this period confusion had been caused by the added presence of *T. grayi* in the guts of wild-caught tsetse. Although this trypanosome develops its metacyclic forms in the hindgut—the "posterior station"—and infects reptiles by contamination with tsetse faeces, after initial ingestion by the fly it first develops in the midgut in the same way as do members of the *T. brucei* group. It was this similarity that caused the confusion among the earlier workers. The life-cycle of *T. grayi* was finally elucidated by Hoare (1931a, b).

It was recognized generally by the workers already cited that human sleeping sickness in the field was caused by what is now named *T. brucei gambiense* Dutton, 1902, spread by *G. palpalis*, though in many animal experiments *T. brucei brucei* had been used, with *G. morsitans* as its vector. Stephens and Fantham (1910) described a different agent of human sleeping sickness, which they called *T. rhodesiense*, now named *T. brucei rhodesiense* Stephens and Fantham, 1910, whose vector was *G. morsitans* and whose characteristics resembled the animal parasite *T. b. brucei* Plimmer and Bradford, 1899. The disease in man was much more acute than that caused by *T. b. gambiense*, and with a different pathology. However, the stages in the fly of these two agents appeared to be identical (Lloyd and Johnson, 1924).

Observations on the establishment of a trypanosome infection in the tsetse fly contained in the first paragraph of the quotation from Buxton (1955) above are derived from Robertson (1912a and 1913). She also noted (1913) that multiplication of trypanosomes occurred soon after ingestion in the mid- and hindguts. After 48 hours she found that the predominant form of the parasite resembled the stumpy form found in the blood of the vertebrate host. By 10 days these forms started to be replaced by longer, thinner forms which moved progressively up the gut towards the proventriculus. After 3 weeks these forms began to invade the salivary glands "from the hypopharynx". Her evidence for this last point rested on finding trypanosomes within the duct but not yet within the gland (Robertson, 1913).

Robertson (1912c) found no intracellular stage or attachment of the parasites to the gut wall, nor did she mention the peritrophic membrane, though this structure had already been reported by Stuhlmann (1907). She observed no sexual phase, but felt that there might well be one, probably in the salivary glands. However, in the second week of some gut infections she did note (Robertson, 1912c) the presence of multinucleate forms and speculated on their function. She dismissed the "male" form reported by Taute (1911) as irrelevant.

C. ROLE OF THE PERITROPHIC MEMBRANE

Thus the main characteristics of the fly infection were established before the First World War, although the final point, namely that salivary gland infections could not occur without previous gut infection, was not made until 1921 by Duke. An important anomaly was the lack of recognition of the role played by the peritrophic membrane. Southgate (1965) drew attention to this curious gap in the story. Robertson (1912b) believed that the absence of trypanosomes from the anterior part of the midgut for 7–12 days after the infected blood-meal was due to their being carried down the gut by the blood of later meals. She envisaged an ebb and flow of multiplying trypanosomes up and down the gut. Observations on the tsetse gut (Southgate, 1965) showed that new blood extends rapidly down to the junction with the hindgut and, before this, there may be forcible evacuation of the products of previous meals: clearly the midgut is an unstable region for the establishment of a large colony of parasites if there is not some method of attachment or special sequestration mechanism available.

The first mention of the peritrophic membrane in relation to development of trypanosomes in tsetse was by Johnson and Lloyd (1929) and Lloyd (1930), who noted that trypanosomes (*T. congolense*) could be found developing in the ectoperitrophic space. Wigglesworth (1929) described the origin of the membrane from an annular pad of proventricular epithelium, "pressed" out from the hardening secretions of those cells. He found it composed mainly of chitin and permeable to digestive enzymes, haemoglobin and haematin, although acting as a "filter" for intact erythrocytes.

Hoare (1931a) showed that the peritrophic membrane was a continuous tube down to the midgut/hindgut junction and first coined the phrases "intra" and "extra" peritrophic spaces, here referred to as "endo" and "ecto" peritrophic spaces. He believed the rectal "spines" disrupted the membrane's posterior end so that its contents could be liberated in the gut lumen.

Taylor (1932) described *T. gambiense* developing in *G. tachinoides* in the ectoperitrophic space. In 1933 Yorke and co-workers showed that within a few days of an infected blood-meal, trypanosomes could no longer be found in the endoperitrophic space—only in the ectoperitrophic space. The trypanosomes then moved up to the proventriculus, later reaching to within its lumen. These workers also believed that the trypanosomes attained the ectoperitrophic space via the torn posterior end of the peritrophic membrane, where it had been ruptured by the rectal "spines" or "teeth".

In 1957 Gordon first demonstrated the passage of trypanosomes across the peritrophic membrane at the upper end of the proventriculus. He cut serial sections of tsetse infected with *T. congolense* and found great numbers in the ectoperitrophic space, but only a few of the same morphological type within the lumen, which he assumed to have just passed through into

the endoperitrophic space. A year later Fairbairn (1958) published photographs of trypanosomes in the proventriculus, where the peritrophic membrane was still "soft", in the act of penetration.

D. OBSERVATIONS CONFLICTING WITH THE TRADITIONAL PATHWAYS

These, then, were the last points completing the cycle quoted from Buxton and shown in Fig. 1a. This complex pathway, involving as it does trypanosomes doubling back on several occasions through different environments within the fly, itself presents problems.

It is probable that its sojourn in the crop gives the trypanosome time to change its metabolism from one involving the use of glucose (in the vertebrate hosts' blood) to its new energy source of proline from the tsetse fly (Harmsen, 1973). The parasite, now a midgut trypomastigote form, enters the peritrophic membrane sac via the proventriculus. Hoare (1931a) and Willett (1966) have clearly demonstrated the peritrophic membrane initially to be a long intact sac that is ruptured or opened only when it reaches the "spines" of the rectum after the fly's first feed (although its proventricular end, as it is being produced from the annular ring of cells (Wigglesworth, 1929), might be soft enough to allow penetration by the parasites). Subsequently the hind end of the membrane remains open throughout the life of the fly. Thus the accepted route would take the trypanosomes into the hindgut itself, taking 3 or 4 days to reach there (Willett, 1966).

Bursell and Berridge (1962) have shown that while the pH of the tsetse midgut contents is around 7·2 (with low osmolarity), the hindgut osmolarity is very high, with a low pH of around 5·8. Thus a trypanosome would have to suffer very dramatic changes in its environment during any circumnavigation of the end of the peritrophic membrane, which it would be unlikely to survive.

This part of the accepted developmental pathway has thus several difficulties. It has been suggested (Wigglesworth, 1929) that the peritrophic membrane could be "inconsistent in the middle section" of the midgut, thus allowing trypanosomes direct access to the ectoperitrophic space: however, Willett (1966) found that, even after a very large first blood-meal, the peritrophic membrane remained intact throughout its length until it finally engaged with the rectal "spines" several days later; this was confirmed by Southgate (1965). Freeman (1970, 1973) suggested that trypanosomes might pass out of the endoperitrophic space via the soft forming peritrophic membrane at the upper end of the proventriculus in the first hour following an infected feed. This represented a reversal of the accepted view of the passage through this area of membrane, which proposed that only mature midgut forms re-entered the endoperitrophic space at this point on their way to the salivary glands.