Tsetse-Fly Physiology*

A Review of Recent Advances and Current Aims

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A review of the literature on tsetse-fly physiology published during the last decade shows that substantial advances have been made in elucidation of the problem of water balance, and in the fields of reproductive and developmental physiology. It is suggested that further studies of reproduction, with particular reference to the reproductive potential of natural populations, are urgently needed; and also that research could fruitfully be directed towards an assessment of the role of starvation as a controlling factor in population dynamics. The author considers that such major projects would stand a better chance of success if they are centred on African research institutes, where a close integration between field and laboratory work can be sustained.

The subject of tsetse-fly physiology was last reviewed by Buxton (1955) in his book The Natural History of Tsetse Flies, in which the literature published up to 1952 was summarized. The data available at that time, despite the fact that they had been obtained under conditions which were usually difficult and with techniques which were often crude, were yet sufficient to give a surprisingly good picture of most aspects of tsetse-fly physiology, at least in broad outline. And it is thanks to the efforts of such early investigators as R. W. Jack, K. and H. Mellanby, P. A. Buxton, C. H. N. Jackson and W. H. Potts, to mention only a few, that a firm basis was available on which to build with later and more refined techniques.

At the time of Buxton’s publication most details of the life-history of the tsetse fly had been worked out, and a considerable amount of data had accumulated on the subject of reproductive physiology; the general pattern of tsetse-fly metabolism had been established, a pattern which differs from that of most Diptera in being based on fat rather than on carbohydrate. The basic processes involved in feeding, digestion and excretion had been elucidated, and the fact that tsetse flies have no other source of water than blood had been established beyond reasonable doubt. Upper and lower temperature limits had been set and certain aspects of behaviour had been investigated. The general problem of water balance had not proved capable of satisfactory elucidation with the techniques then in use, and the subject was accordingly in a somewhat controversial state.

In the years which followed, tsetse-fly physiology became the subject of rather more systematic investigation, partly in East and West African laboratories, partly at European research centres. As a result it has been possible to add many details to the outline picture delineated in Buxton’s book, but much remains to be done. For purposes of review it will be convenient to consider the advances made under a series of separate headings.

REVIEW OF THE LITERATURE, 1952-62

Reproduction

A comprehensive study of reproduction in laboratory-reared females of Glossina palpalis was made by Hoffman (1954), and served to confirm a number of earlier observations on the ovulation cycle and on larval development in general. Of particular interest were descriptions of the cyclical activity of the milk gland epithelium, which was shown to be secreting actively only during the middle period of intra-uterine development. This is clearly of relevance in relation to the importance of timing in the feeding of pregnant females; blood must be

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available during the early stages of pregnancy if it is to benefit the larva.

The limited secretory span of the milk gland was confirmed by Bursell & Jackson (1957), who added some further observations on the intra-uterine feeding of the larva and described cyclical changes in the development of the choriothete. The degenerative changes which occur in this organ following removal of the first-instar exuvium are clearly of importance in relation to the general physiology of pregnancy in so far as materials resorbed during muscular and glandular atrophy may become available as raw material for the elaboration of milk gland secretion.

Recent work by Saunders (1960a) has rectified an error perpetuated by a succession of workers since the earliest investigations of tsetse fly reproduction; he has shown that each ovary contains two ovarioles, not one as had hitherto been supposed; and that although a regular alternation between right and left ovaries characterizes successive pregnancies, the nature of this alternation is a little more complex than had previously been thought. On the basis of this finding and the occurrence of so-called “funicle stumps”, evidence of previous ovulation, the author describes a method for determining the physiological age of female tsetse flies up to the age of 40 days (Saunders, 1960b, 1960c).

In addition to these investigations of particular aspects of reproductive physiology, a great deal of information has accumulated in connexion with general projects involving the laboratory culture of tsetse flies (e.g., Willett, 1953; Foster, 1957). But unfortunately studies of this kind, although they may achieve their object of providing improved methods of maintenance, tend to raise problems rather than solve them, and the results obtained can be interpreted with certainty only when more is known of the basic details of reproductive physiology.

There is no doubt, then, that important additions to our knowledge of reproduction in tsetse flies have been made during the last ten years. But we are still very far from a complete understanding of the processes involved; and, above all, such results as have been obtained cannot be applied to the fly in its natural state with any degree of confidence. Most workers have been careful to comment upon the prevalence of small pupae in laboratory-maintained stocks; on the frequency with which larvae tend to be aborted; on the irregularity of interlarval periods; and on the fact that the rate of reproduction is usually far below the theoretical maximum. The discovery by Saunders (1960c) that degeneration of egg follicles may occur, thus leading to intermittent larva production, is of obvious relevance here. But it remains uncertain to what extent phenomena of this kind are characteristic of the fly in its natural habitat.

Development

 Larval. The morphogenesis of the tracheal system of the tsetse larva was described by Bursell (1955) and the peculiar respiratory lobes which characterize the third-instar larva were found to represent special adaptations to intra-uterine respiration.

 Pupal. The effect of temperature on the consumption of fat during pupal development in different species of Glossina has been investigated (Bursell, 1960c), and the occurrence of an optimum temperature at about 25°C was demonstrated; at this temperature the amount of fat required to complete pupal development is minimal; at higher or lower temperatures the total amount of fat consumed increases with the result that the teneral fly emerges with smaller fat reserves. It was also shown that the smaller species, and within a species the smaller individuals, have relatively smaller fat reserves at the time of pupation. In view of these results it was suggested that the distribution of species like G. morsitans and G. swynnertoni might be limited by exhaustion of fat reserves during development under extreme climatic conditions.

Temperature was also found to affect the size of flies emerging from pupae of a given weight (Bursell, 1960a), any increase above 28°C resulting in a marked reduction in the size of emerging flies.

Developmental abnormalities in respect of wing venation have been investigated by Glasgow (1960). The occurrence of stub veins was found to be most common in the hot season, and the frequency of some of the variations could be increased experimentally by subjecting pupae to high temperatures at early stages of pupal development. Glasgow suggests that the temperature effect might be calibrated to give information about the temperature of natural breeding-sites. But it is probable that such information could be gained more accurately and easily by other means, especially in view of the absence of any correlation between temperature and aberration frequency reported by Welch (1960).

The temperature and the humidity experienced during pupal development were found to affect the
extent of pigmentation of the dark abdominal banding in *G. pallidipes* but not in other species studied (Bursell, 1960b). After development in saturated air the bands are heavily pigmented on all abdominal terga, while at 20% relative humidity only parts of the fourth and fifth terga show heavy darkening. Where the temperature of pupal sites remains below 28°C the extent of pigmentation provides a sensitive indication of the humidity to which the pupae have been subjected.

**Post-teneral.** A massive development of thoracic musculature was found to occur after emergence of tsetse flies from their pupae, the process extending over the first two or three hunger cycles in the field (Bursell, 1961c). Subsequent histological studies have shown that the development represents an increase in the size of existing muscles, not a laying down of new ones (Glasgow & Glasgow, 1962).

**Water balance**

The water balance of tsetse flies has been the subject of intensive investigation during the period under review (Bursell, 1957b, 1958, 1959, 1960d) and the results may be discussed under two headings.

**Pupal water balance.** During pupal development water is lost by transpiration and by excretion; in both cases the amounts lost are subject to active regulation so that if water reserves have been seriously depleted the rates of transpiration and of excretion are reduced. These powers of active control are developed to a similar degree in all the species studied, but they operate against a background of puparium permeabilities which differ greatly. These differences, together with the ability of certain species to tolerate dehydration better than others, result in a wide spectrum of resistance to desiccation, ranging from species like *G. longipennis*, which can tolerate exposure to a relative humidity of zero for the whole of the pupal period, to *G. brevipalpis*, in which pupal mortality increases progressively as the relative humidity falls below 80%. A detailed correspondence exists between the degree of resistance to desiccation and the habitat in which the pupae occur, and on the basis of this correspondence it was concluded that the water balance of pupal stages may constitute an important limiting factor in the distribution of species.

**Adult water balance.** The adult, like the pupa, shows considerable powers of regulation as regards both excretory and transpiratory losses of water. The amount lost by transpiration is controlled by spiracular closure in relation both to the animal’s water content and to the ambient humidity; the drier the air and the more seriously depleted the water reserves of the insect, the more stringent the control. The water lost by excretion and defaecation is also regulated, presumably through the activities of the rectal glands, in such a way that losses are minimized when desiccation threatens.

A comparative study of eight East African species of tsetse fly failed to reveal any major differences in resistance to desiccation, nor was there any indication that such small differences as could be demonstrated were in any way related to the habitat of the species concerned. This general lack of interspecific variation is in accord with earlier work of Geigy & Huber (1952), who failed to find evidence of differences in the structure and mode of operation of spiracles in five species of tsetse-fly.

It seems, then, that the habitat requirements of different members of the genus must be interpreted in terms of differences in the powers of resistance to desiccation of early developmental stages rather than of the adult fly. But this leaves the mechanism of the regulation of population numbers within the distribution limits still in question. Buxton (1955) devotes a considerable amount of space to discussions of the correlation between fly numbers and saturation deficit which is so regular an item of field experience. It seemed reasonable to expect that the correlation might be a causal one, as indeed it has been considered by many; in fact that during the hot dry season death by desiccation makes an important contribution to mortality. But this view does not fit well with the occurrence of extremely well-developed powers of regulation, nor with the lack of specific differences in resistance to desiccation. In an attempt to decide the question a more direct approach was made through an analysis of the physiological state of flies captured in the field at different times of the year and in different parts of East Africa (Bursell, 1961a). The results indicated that death by desiccation, even in species occupying xerophytic habitats and at the height of the dry season, was not a common occurrence, and that starvation was a much more potent cause of mortality. The same conclusion emerges from earlier work with teneral flies (Bursell, 1959).

Seen as a whole the results of these investigations of the general problem of water balance constitute very strong evidence against the view that death by desiccation is an important factor in tsetse-fly biology. It must be concluded that the regularly
observed correlations between saturation deficit and tsetse-fly numbers is a fortuitous one—that fly densities are low when saturation deficits are high but not because saturation deficits are high.

**Metabolism**

Investigations of tsetse-fly metabolism during the past ten years have been of a rather fragmentary nature, and no great advance can be said to have been made in the field as a whole. The work of Fairbairn & Williamson (1956) and of Williamson (1956), undertaken with specific reference to the invasion of salivary glands by polymorphic trypanosomes, has provided data on the composition of the salivary secretion in respect of free amino acids, sugars and proteins. Studies in Switzerland (Geigy et al., 1959), also carried out with reference to trypanosome infections, have demonstrated the presence of minute quantities of the disaccharide trehalose in the tsetse-fly (between 0.1 μg and 1.0 μg per fly, compared with values of 150 for the blow-fly); glucose was present in small amounts and glycogen absent. These results are in accord with concurrent analyses in East Africa (Bursell, 1960e), which showed negligible quantities of glycogen and reducing sugars. Clearly the function of carbohydrates in the tsetse fly is not that of a metabolic reserve as it is in many other insects. On the other hand, substantial quantities of free amino acid occur in the tsetse-fly thorax, the amount of alanine in particular decreasing greatly in the course of starvation. Further work will be required to determine the possible role of these substances as expendable food reserves.

Attempts have been made to improve assessments of the physiological condition of flies caught in the field by including in the analysis an estimate of the quantity of residual blood meal (Bursell, 1961b), and this technique has been applied to studies of the rate of digestion of the blood meal under natural conditions (Harley & Pilson, 1961) and of the seasonal fluctuations in the nutritional state of *G. swynnertoni* (Glasgow & Bursell, 1961). Nutritional stresses in the population of *G. swynnertoni* were found to be correlated with a decrease in the size of flies in succeeding generations, presumably through an effect on the pregnant female.

The effect of climate on the frequency of feeding has been studied by Jackson (1954) and, using a more direct technique, by Glasgow (1961); the results agree in showing that during the hot dry season the interval between meals is about three days, but the effect of season appears to be smaller than would have been supposed on the basis of the earlier work.

**Behaviour**

A considerable amount of work has been done on various aspects of behaviour during the last ten years, involving studies of the reaction of flies to simple stimulus situations as well as observations on the behaviour of flies in the field.

The effect of humidity on the activity of different species of tsetse-fly has been investigated (Bursell, 1957b); the insects were found to exhibit a marked orthokinesis, with the frequency of movement twice as high in dry as in saturated air.

Studies on the reproductive behaviour of *G. pallipes* have been made by West African workers (Nash, 1955; Jordan, 1958). The results show that males less than 24 hours old tend to be impotent, their virility increasing progressively during the first week of life. Virgin females mate most readily during early life, becoming progressively less ready to mate after the third day. Previously mated females are less willing to copulate than virgins at all times, and in females older than 10 days remating is rare.

The selection of breeding sites by larvipositing females of *G. pallipes* (Parker, 1956a) was found to involve attraction to black objects or patches of shade; attraction to rough surfaces and to relatively dry soil; and avoidance of sites which were more than 3.5°C cooler than ambient air. These results are in accord with what is known about breeding under natural conditions. They are relevant also to observations on the newly deposited larva, which was found to burrow more readily into rough-surfaced and relatively dry soil than into smooth and damp soil (Parker, 1956b). In *G. pallidipes* temperature appears to affect the depth to which the larvae burrow (Bursell, 1960b), in accord with observations that puparia are generally found at greater depths during the hot than during the cool season.

An investigation of the responses of *G. mediterraneum* to different stimuli was made in an attempt to discover the stimuli involved in host-finding (Chapman, 1961). Activity could be induced by the movement of a black screen, and the flies showed some measure of orientation to the moving object. A kinetic effect of certain odours was demonstrated in confirmation of earlier experiments by Hughes (1957a, 1957b), who showed that activity could be increased by a variety of chemical vapours.
Coupled with the marked up-wind orientation of *G. medicorum* in a wind tunnel these reactions to smell might assist in the location of host animals. But the fact that in none of these experiments could a difference in response be established between early and late hunger stages casts some doubt on the relevance of the experiments to the problem of host-finding.

The responses of tsetse flies to cloth screens in the field was investigated by Barras (1960), who found that a preference was shown for darker screens and that disruption of the black surface by white stripes decreased its attractiveness. Here again an interpretation of the results is made difficult by uncertainties regarding the composition of the catch. The value of such work would be enormously increased if it were coupled with information about the physiological state of the flies attracted.

That differences in physiological state are associated with marked differences in behaviour has been shown by Bursell (1961b), who on the basis of an analysis of the fat content and the stage of digestion in flies caught by different methods suggested that four phases of behaviour could be considered to follow one another in the course of a hunger cycle—a phase of inactivity; a phase of sexual appetitive behaviour; and two phases of appetitive behaviour relating to the feeding reaction, during the first of which flies are unresponsive to moving objects, and during the second of which they are attracted to moving objects.

Glasgow (1961) found that tsetse flies showed a preference for feeding on shaded bait rather than on bait exposed to the sun. And Dethier (1954) investigated the reactions involved in the act of probing, finding that heat is the primary stimulus in eliciting the normal biting response, acting primarily through antennal thermo-receptors. The mechanics of proboscis penetration and the tapping of blood have been described by Gordon et al. (1956).

The identification of blood meals by precipitin testing has an obvious bearing on the general problem of tsetse-fly behaviour, but the results need not be considered here as they are discussed in detail in another paper.1

**AIMS**

The past ten years have been fruitful in furthering our knowledge of tsetse-fly physiology along a fairly broad front. But at most points there remains a most unfortunate gap between what is known about the physiology and behaviour of the tsetse fly in the laboratory and what may be surmised about its physiology and behaviour in the field. There can be little doubt that the difficulties involved in bridging this gap will be accentuated by any tendency to shift the centres of research from Africa to Europe. Not only would such a shift preclude a regular traffic of men and ideas between the two disciplines, but it would involve reliance on material bred and maintained entirely under laboratory conditions. The dangers of such reliance have often been pointed out; the list of deficiencies shown by laboratory-maintained flies is a long one and well known—subnormal fat contents, subnormal reproductive capacity, subnormal pupal weights as well as a number of less palpable abnormalities—and to it must now be added the inability to complete post-teneral development in respect of flight musculature (Bursell, 1961c). To embark upon research projects with such experimental material is to study abnormal physiology and abnormal behaviour, and unless constant recourse can be had to checks with insects captured in the field, conclusions reached may have little relevance to the problem of tsetse fly biology. Objections of this kind may admittedly be raised against any experimental work that involves isolation of animals from their environment; but they carry very much greater weight when such animals are manifestly abnormal in respect of the very objects of study—metabolism, reproduction, behaviour. It is possible that with further research into methods of maintenance a closer approach to normality may be achieved, but even then it would probably be more economical and certainly more conducive to liaison between laboratory and field to base research on regions where flies can be obtained from their natural habitat. This is not to question the very great value of the research done in Europe, only to recognize its limitations where what is aimed at is a comprehensive attack on particular aspects of tsetse fly biology.

Within the field of tsetse-fly physiology two aspects would seem to stand in urgent need of detailed investigation, as much for the insight which such studies would provide into key points of the biology of the tsetse fly as for their practical importance—one the problem of reproduction, the other that of starvation. Both exemplify in the highest degree the necessity for a close integration of laboratory and field work.

As was pointed out earlier, a considerable amount of data is available concerning the broad features of

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1 See the article by B. Weitz on page 711 of this issue.
reproductive physiology and behaviour. But we know as yet nothing about the endocrine control of reproduction, nor to what extent or by what means the controlling mechanisms come under the influence of environmental factors and the nutritional state. Above all, we have no idea in what way or to what extent the results of laboratory investigations may be applicable in the field. For purposes of calculating the reproductive potential of tsetse-fly populations, it is usual to assume (Yeo & Simpson, 1960) that the modal value for interlarval periods under conditions of laboratory maintenance may be taken as applicable to natural populations. But there is little justification for this assumption, and, in view of the marked influence of feeding frequency on interlarval periods (Foster, 1957) together with the fact that starvation may be common in natural populations (Bursell, 1961a), it is a questionable one.

Accurate assessment of reproductive potential in tsetse flies must stand as the very cornerstone of an understanding of their biology, and investigations of the problems involved are of corresponding importance. Such assessment would in addition lend badly needed precision to theoretical treatments of population dynamics as involved in the planning of control measures and evaluation of their effects.

If the birth-rate of tsetse flies is a matter for urgent investigation, the death-rate is no less so. Since desiccation can no longer be considered an important cause of death, other explanations must be sought for the heavy mortality which usually characterizes the hot season; and there is evidence that starvation may be a likely alternative. In the first place selection for size in natural populations has been demonstrated in a number of species (Jackson, 1948; Bursell, 1959; Bursell & Glasgow, 1960; Glasgow, 1961), and this can most reasonably be attributed to death by starvation of the smaller teneral flies with their relatively scant fat reserves. Secondly, it has been shown that a substantial proportion of flies in the normal habitat have fat contents close to or within the normal range of lethal limits and must live in danger of imminent starvation (Bursell, 1961b).

An investigation of the extent to which exhaustion of fat reserves contributes to the total mortality of a tsetse-fly population would obviously be of the greatest theoretical and practical importance, particularly in relation to the wider problem of the relation between the fly and its hosts. Here the ground has been prepared by the work of Weitz, which undoubtedly represents the most important advance in tsetse fly biology which the period under review can boast. Unfortunately the tendency has been to admire the results of this work rather than to follow up their implications, implications which are of far-reaching significance in relation to the complex of interactions between habitat, host and fly, and hold, perhaps, the key to an understanding of the biology of the tsetse fly.

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From the physiological point of view what would be required as a complement to investigations on the relation between host and fly is a detailed study of intermediary metabolism. Without this a full assessment of nutritional state is impossible, and without that the problem of starvation will remain unanswered. Account must be taken not only of fat reserves, amino acid reserves and reserves represented by the residual blood meal, but also of the nature and extent of interconversions between the different types of reserves and thus of what one might term their content of available energy. Clearly a milligram of residual blood meal will not have the same content of available energy as a milligram of fat, since a part of its energy would be lost in digestion, absorption, deamination and excretion. Only through a fundamental investigation of intermediary, excretory and respiratory metabolism could the total reserves of the fly be accurately estimated, and only when this can be done will it be possible to put the problem of the role of starvation in population dynamics on a quantitative basis.

RÉSUMÉ

L'auteur passe une revue des acquisitions faites depuis 10 ans en matière de physiologie de la mouche tsé-tsé. Des progrès substantiels ont été réalisés en ce qui concerne la physiologie de la reproduction, mais l'on sait encore mal dans quelle mesure les données du laboratoire peuvent être appliquées à la vie normale de la mouche. Certains aspects de la physiologie de la croissance ont été étudiés, et l'existence de rapports entre la température et la consommation de graisse pendant le stade de chrysalide incite à penser que les températures hivernales basses peuvent freiner la vie à ce stade du développement. Le bilan hydrique au cours des premiers stades et chez la mouche adulte a fait l'objet d'une série de recherches. Il en ressort que la distribution géographique de l'espèce dépend de la résistance à la dessiccation lors des premiers stades mais que la dessiccation n'est pas, dans les condi-
tions habituelles, une cause fréquente de mort de la mouche adulte. Les résultats des recherches sur les différents aspects du comportement de la mouche tsé-tsé sont décrits.

Pour permettre une interprétation de la dynamique des populations de la tsé-tsé, il est de la plus haute importance de tacher de combler le fossé existant entre ce que l'on sait de la reproduction en laboratoire et ce que l'on soupçonne de la reproduction dans la nature. De même, il est essentiel d'aborder de front le problème de la mortalité des mouches dans les conditions naturelles. Il semble que le jeûne soit une cause importante de décès, mais une appréciation quantitative nécessite des recherches ultérieures sur le métabolisme, parallèlement à des investigations portant sur le rapport entre la mouche tsé-tsé et ses hôtes dans les conditions naturelles. Il semble bien que l'un comme l'autre de ces programmes ne puissent être accomplis que si la recherche est effectuée dans un centre africain. C'est le seul moyen de réaliser une synergie parfaite des études de laboratoires et des enquêtes sur le terrain.

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