BEHAVIOURAL CHANGES AND THEIR CONTROL DURING STARVATION IN THE TSETSE FLY *GLOSSINA MORSITANS*

by

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To Bill

Abstract

An investigation into physiological parameters potentially involved in a causal relationship with known changes in responsiveness of Glossina morsitans across starvation was made. For mature flies, osmotic pressure of the haemolymph, the degree of crop stretch at the last meal, the amount of diuresis and the length of time feeding did not change with starvation in the same way as, or manipulation of them had no effect on, spontaneous locomotor activity. For tenerals, crop stretch with air at emergence raised the threshold to locomotor activity. For mature flies, it is proposed that a common shortage of proline immediately after a meal accounts for the observed base line level of activity. Subsequent activity levels reflected the weight of nutrient reserve remaining.

Fly weight was known to correlate with changes in spontaneous locomotor activity. Wings of flies were not involved in monitoring weight, but legs were sensitive to different weights and it is concluded that they are responsible for monitoring weight.

Investigations into behavioural changes with starvation showed that a wider range of temperatures elicited probing as starvation increased. Probing responsiveness continued to increase until death. Visual responsiveness was greater immediately after a spontaneous activity burst than mid-way between two such bursts, and continued to increase until death.

Observations revealed activity bursts to consist of alternating bouts of flight and rest interspersed with bouts of clean and walk. There appeared to be an inverse relationship between the number of activity and cleaning bursts which is examined and discussed in terms of competition and suitable levels of arousal. An increase in volume of the apparatus housing the fly resulted in fewer, longer, bouts per activity burst, whilst cleaning bursts were unchanged.

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CHAPTER 1

1. General Introduction

The tsetse fly Glossina morsitans (Westwood) is an obligatory bloodsucker, inhabiting environments in Africa which are often arid. It is typically found in woodland savannah, and its occupation of the wooded grasslands of West, Central and Eastern Africa enable this fly to transmit nagana on a colossal scale, whilst it is also the main carrier of Rhodesian sleeping sickness.

Within its habitat, trees and shrubs provide perching places for recuperative rest, shelter from adverse climates and predators, as well as vantage points from which to view a potential host (Ford, 1970). Many workers have reported seasonable habitat preferences of G. morsitans, e.g. Pilson and Pilson (1967), showed that males at least, were especially abundant in riverine vegetation during the hot dry season, and more abundant in drier woodland at other seasons. There is also a seasonal shift in the breeding grounds chosen by the female for the act of larviposition. In the rains larvae would be deposited under logs, after the rains in scattered thicket sites and during the hot dry season on the floor of the most dense parts of the forest islands, (Nash, 1969).

G.morsitans typically occurs in areas where game is plentiful and the human population density is low. The fly takes its bloodmeals notably from suids and bovids, and especially from warthog among the suids (Nash, 1969, after Weitz). The flies form a following swarm which moves after a large slow-moving game animal. Early work had shown the swarm to be composed largely of males, with small numbers of young flies which feed if the host stops, and the remainder of old flies which do not feed,

but will mate with a female if the opportunity occurs. (Buxton, 1955). Recent work (Vale, 1972) using vertical electric nets carried some distance behind a mobile bait gave a higher proportion of females, so that there may also be a more diffuse swarm of females associated with a host.

Biting in the field occurs in diel patterns of activity (Pilson and Pilson, 1967), and laboratory studies (Brady (1972a), and Brady and Crump (1978), have shown this rhythm to be approximately 80% endogenous. These workers comment on the adaptive value of the rhythm rendering the fly active at the same time as the warthog host. G.morsitans seems to be mainly an opportunist feeder, feeding when a suitable host is encountered, and Bursell, (1966b) found flies feeding within the range of 10-80% of full nutritional reserve load remaining.

Visual contact has long been considered important in location of a host suitable for a bloodmeal. Chapman (1961) showed flies to be responsive to a 120 cm x 90 cm black target moved across the field of vision at a distance of 50 yards. In a laboratory study Gatehouse (1972a) found unfed teneral flies to be responsive to a visual stimulus, and that in the males the response was increased in the presence of calf odour. Fieldwork by Dean et al. (1969b) suggested that attraction to the host was visual as few individuals of G.morsitans found oxen concealed by screens, whereas Vale (1972) found that most flies were caught on the downwind side of an electric pen enclosing a bait-ox, suggesting that in this instance windborne odours may have been involved in host location. Brady (1972b) in a laboratory study, measured response to a black stripe visual stimulus, and found an exponential increase in responsiveness across four days of starvation.

In the field it has been shown that host preference also shifts with an increase in starvation. Ford (1969a) showed that flies which probed man had less fat reserve than those which probed an ox. Flies which probed man were also younger than flies probing an ox, and it is advantageous for host preference to be less strong in unfed teneral flies in view of the need for these flies to find the first meal quickly, (Bursell, 1960b).

To quantify further the field observations of fly behaviour, and to explain the behavioural changes with starvation in physiological terms, much laboratory research has been conducted. This work also enabled an assessment to be made of the impact on behaviour of sterilisation, so that the optimum dosage for sterilisation of males for release as a method of control could be found (Langley et al., 1974). Detailed laboratory research has also been conducted into many other aspects of tsetse fly physiology, especially those associated with feeding requirements for successful in vitro culture. Table 1.1. gives a list of the principal contributors to topics of research other than those discussed in detail with reference to feeding behaviour.

The behaviour studies have revealed the following. Spontaneous locomotor activity of Glossina morsitans was measured in actographs, and was shown to increase exponentially for five days after the last meal in mature female and male flies, and to increase for four days after emergence in teneral flies. The changes are affected by modulation of periods of inactivity, whilst the burst length remains the same. (Brady, 1972a). Visual responsiveness, measured by number of take offs stimulated by moving objects, was shown to increase exponentially for four days after emergence in all teneral flies and for five days after the last meal in all mature flies. The intensity of orientation to the visual stimulus also increased with starvation, at least in teneral males. Human odour elicited take-offs in the absence of visual stimulation, and enhanced the visual responses

Table 1.1. List of research topics and authors

Topic	Author
Reviews of studies of physiology and feeding requirements of tsetse and other haematophagus insects	Bursell et al., 1974; Friend & Smith, 1977; Galun, 1975b; Langley, 1977; Rice, 1972a.
Requirements for successful *in vitro* culture for mass rearing.	Langley, 1966b, 1972; Langley & Maly, 1969; Langley & Pimley, 1973; Mews *et al.*, 1976; Mews *et al.*, 1977.
Monitoring of effects of long-term laboratory rearing on fecundity and field performance	Dame *et al.*, 1975; Jordan *et al.*, 1970; Vale *et al.*, 1976.
Feeding and digestion	Langley, 1966a, 1967a & b, 1970.
Digestive enzymes	Gooding, 1974b & d, 1975.
Chemical factors affecting engorgement.	Galun, 1975a; Mitchell, 1976b; Mitchell & Reinouts van Haga-Kelker, 1976.
Crop emptying and meal size regulation	Moloo & Kutuza, 1970; Tobe & Davey, 1972a.
Control mechanism of diuresis, and factors affecting it	Gee, 1975a & b, 1976, 1977; Tobe, 1974.
Metabolism and uses of, the bloodmeal.	Bursell, 1963, 1966; McCabe, 1973.
Structure, function, and innervation of the gut.	Langley, 1965; Finlayson & Rice, 1972; Rice, 1970a,b,c & d, 1972a & b.
Mouthpart sensillae and their function	Rice *et al.*, 1973a & b.
Water balance	Bursell, 1957, 1959a, 1957b, 1961.
Change in salivation with starvation	Youdeowei, 1975a & b.
Flight metabolism	Bursell, 1978; Hargrove, 1975a & b, 1976.
Sound production	Kolbe, 1974.
Sex recognition pheromone	Langley & Pimley, 1975.
Volume relationships during pregnancy	Tobe & Davey, 1972a & b.
Nutrient transfer during pregnancy	Moloo, 1976a & b, 1977; Tobe *et al.*, 1973.

if these were tested within three minutes of the start of odour stimulation, (Brady, 1972b). The number of matchstick-mounted flies which would probe a warmed foam rubber ball was shown to increase linearly for four days after the last meal in mature flies, and for three days after emergence in tenerals. The time taken to respond to the stimulus fell considerably across starvation (Brady, 1973). The temperature threshold for skototaxis was shown to decrease with starvation in female flies, (Huyton and Brady, 1975).

Defaecation frequency, and with the exception of probing, all responses described above are strongly modulated across the photophase of LD 12:12 in the V pattern typical of biting behaviour in the field. Morning and evening responses were greatest, and noon least. There is a more subdued modulation of the probing response. The rhythms of spontaneous locomotor activity and visual responsiveness persist in constant conditions, indicating that the underlying rhythm has a largely circadian base. (Brady, 1975). Brady and Crump (1978), in further activity studies showed a bimodal response to temperature, and with a reanalysis of published field data concluded that some 80% of the V pattern of biting activity in the field is due to an endogenous circadian rhythm and only 20% to direct control by temperature.

Changes in responsiveness with starvation have also been shown in the Blowfly Phormia regina, e.g. Dethier and Rhoades (1954) demonstrated a ten-million-fold change in acceptance threshold to sucrose with starvation. The amount of a standard sugar solution which is ingested has been shown to increase with deprivation time (Gelperin, 1966a). Barton Browne and Evans (1960) showed that spontaneous locomotor activity, measured as passage through a funnel-connected series of four boxes, increased with starvation. Green (1974a) showed that spontaneous locomotor activity measured in actographs increased exponentially with starvation in both tenerals and fed flies. As in tsetse flies,

again the change was affected by a modulation of periods of inactivity.

Whilst the mechanisms controlling feeding thresholds for the blowfly have largely been elucidated (Dethier, 1969; Barton Browne, 1975), spontaneous locomotor activity and taste threshold do not change in the same way with starvation, (Barton Browne and Evans, 1960). Evans and Barton Browne, (1960), considering the possible mechanisms of control, concluded that blood dilution or the rate of crop emptying may be the factors involved. Green (1964b) found that activity level did correlate with the rate of crop emptying, and that when the immobile member of a parabiotic pair was fed, the activity of the mobile member was reduced. He suggested that the corpora cardiaca released a hormone to inhibit activity when the foregut receptors were stimulated by the movement of food pellets. Barton Browne (1975) in a review of the evidence suggested that activity may be related to haemolymph composition.

In Locusta migratoria, if the osmotic pressure of the haemolymph is experimentally increased 20 minutes before a meal, the volume of the meal is reduced, (Bernays and Chapman, 1974c). However, many insects regulate the osmotic pressure of their haemolymph (Florkin and Jeuniaux, 1964), e.g., by lowering the concentration of solutes to compensate for reduced haemolymph volume during dehydration, as shown in Chortoicetes terminifera (Djajakusumah and Miles, 1966).

Tobe and Davey (1972a and b), had shown that the haemolymph volume of female G.austeni remains at 5ml across larviposition, and was not affected by feeding. However, no data were available to show if the osmotic pressure of the haemolymph of G.morsitans remains constant during starvation, or if it alters in such a way that a causal relationship between it and activity level may be inferred. As a preliminary step to investigate this possibility, the osmotic pressure of the haemolymph was monitored across starvation. (Chapter 5).

Abdominal stretch receptors had been shown by Green (1964b), to be not involved in control of activity of Phormia. However Brady (1975), showed that of several plausible parameters which tsetse flies might be measuring across starvation to control the observed changes in behaviour, abdominal weight, or whole fly weight correlated most strongly with the change in spontaneous locomotor activity. This correlation was improved by correcting for weight at emergence, and Brady concluded that the fly's behavioural thresholds are modulated by information about its weight or abdominal volume related to a base line set at eclosion. The weight reflects the fly's nutritional state. That tsetse flies monitor their weight or volume is further indicated by Tobe and Davey (1972a), who found that female G.austeni feed to a constant weight irrespective of the weight of the larva.

With such strong evidence of a causal relationship between weight and behaviour, it was decided to investigate weight sensitivity of the two most plausible weight receptors, the legs and the wings (Chapter 4). The weight sensitivity of the legs to small balls of different weight was monitored across starvation, to see if it changed in a parallel way to real changes in weight, i.e. in parallel to what the fly's legs would 'expect' to hold. The weight sensitivity of flies with and without feedback from their own weight was compared to see if such feedback influenced the change in weight sensitivity. The effect of a change in the real weight of the fly on weight sensitivity of the legs was also investigated by feeding. Wing weight-sensitivity was investigated by monitoring the effect of an apparent change in load on the wings on activity level. From these experiments it was hoped to show if one of these possible weight receptors is responsible for relaying information concerning the fly's weight and nutritional state to the CNS, and ultimately setting the activity level.

Brady, (1975) had shown that flies which reach a peak of activity sooner than their peers also go into decline sooner, and thus the behaviour is influenced more by the state of the flys' reserves than by the temporal relation to the last meal. Hudson (1958), in an investigation of the mechanism controlling the threshold to glucose in Phormia regina showed that enforced flight causes a fall in threshold, whilst in control flies thresholds are not altered during the same period of time. This indicates that for Phormia also, the state of the reserves is more important in setting thresholds than the effects of the feeding act and the length of time since the meal. Barton Browne (1975), concluded from this, and from the time lag between the act of feeding and the rise to maximum, threshold (neural effects from the act of feeding would be expected to take place straight away), that in Phormia there are no long lasting neural effects from the act of feeding.

Whilst this had also been implied for the tsetse (see Brady, (1975), above), it was decided to obtain a clearer picture of any central effects of the act of feeding. This was investigated by separating the sensory input from the sequence of behavioural events involved in feeding from the sensory input of increase in nutrient reserves resultant to a meal, by feeding partial meals. (Chapter 7). Hopkins (1964) had shown that in Stomoxys calcitrans, partial feeds of half the normal size had no immediate effect in altering the threshold to two different vapour sources, although measurements were not made of the threshold at different times after the partial meal.

Brady (1975) noted that stretch receptors in the crop of tsetse flies are unlikely to be involved in the change of activity after the meal since in G.brevipalpis the crop is completely empty within twenty minutes of feeding (Moloo and Kutuza, 1970). However, the role of crop expansion in tenerals had not been investigated.

Cottrel (1962), described the imaginal ecdysis of another dipteran, Calliphora erythrocephala. Wing extension and 128% increase in volume are achieved by passing air into the midgut by action of the cibarial pump. Langley (1967b) showed that prevention of crop expansion during ecdysis in G.morsitans by puncturing of the ptilinum, prevented the usual post emergence rise in midgut protease enzymes, as well as preventing expansion and wing extension. Crop expansion is thus apparently involved in release of midgut enzymes in tenerals, and is a common stimulus preceding the similar change in activity of teneral and mature flies shown by Brady (1972a). It was thus decided to investigate if prevention of crop expansion by puncturing the ptilinum of newly emerged flies would have any effect on subsequent activity levels in teneral flies (Chapter 6).

It has been shown on many occasions that tsetse flies can be induced to imbibe diluted blood (Yorke and Blacklock, 1915; Galun and Margalit, 1969; Langley, 1966a), or non-nutritive solutions if the response is enhanced by the presence of adenine nucleotide phagostimulants (Gee, 1976; Galun and Margalit, 1970; Langley, 1972), but these studies were concerned with the feeding response itself, or in one case with the effect of the meal composition on diuresis, and no data were available on the effect of imbibition of dilute blood on activity across starvation. It was decided to investigate this by giving different flies similar volumes of different dilution. This would give all flies the same length of time feeding and the same degree of crop expansion. Those flies fed on more dilute blood would have more extensive diuresis and would have less nutrient remaining and would gain less weight from the meal. It was hoped that this experiment would indicate which parameters were most important in influencing post diluted meal behaviour (Chapter 6).

The series of experiments just described was thus designed to investigate any role of blood osmotic pressure, the weight of flies, and the various sensory inputs from the act of feeding, in influencing the level of spontaneous locomotor activity pursuant to a bloodmeal. Further to these attempts to elucidate control mechanisms for observed changes in spontaneous locomotor activity, additional investigations were made into the behavioural changes themselves. Probing responsiveness was monitored across starvation, and recordings were made of observations of changes in spontaneous and stimulated locomotor activity, so that all activity could be monitored, and not just flight as recorded by rocking box actographs.

Probing responsiveness has been shown by Dethier (1954) to be influenced by temperature, and he concluded that the main site of reception was the antennae. Langley (1972) found that removal of the antennae of adult females caused a reduction in frequency, but not a complete abolition of feeding. Reinouts van Haga and Mitchell (1975) found that removal, or treatment with glacial acetic acid, of the prothoracic legs caused a reduction in or an elimination of the probing response. They concluded that in the tsetse fly probing response to temperature, a secondary input from receptors on the tarsi of the prothoracic legs augments the primary input from the antennae.

Brady (1973), by offering tethered flies a lightweight foam plastic ball of unknown, but substantially higher than ambient, temperature to hold at intervals, showed that the responsiveness of mature and teneral male flies increased linearly across starvation. The increase continued until days 4 and 3 respectively. In addition to an increase in the numbers of flies probing with starvation, there was a progressive reduction in the time taken to probe which can be interpreted as an increase in responsiveness of each fly as its starvation increased.

Work on blowflies, (Dethier and Chadwick, 1948), had shown that the chemosensory stimulation needed to elicit proboscis extension decreased with deprivation time.

The present study was undertaken to find which of a range of known temperatures offered was most suitable for probing response in tsetse flies, and to investigate if the response to this temperature, and to temperatures above and below it alters in the same way across starvation.

Consideration of the following resulted in recordings of observations of behaviour being made. It was known that in mature male tsetse flies, spontaneous locomotor activity (Brady, 1972a, 1975), visual responsiveness (Brady 1972b), and probing responsiveness (Brady, 1973), increased with starvation. Brady (1975b) suggested that it would be economical if the central processes which controlled the circadian modulation of behaviour also controlled the changes with starvation. Whilst actograph results (Brady, 1972a) had shown that spontaneous flight was performed in bursts of ca. 1 minute in duration whose length did not change with starvation, nothing was known about other behaviours performed spontaneously, e.g., walking and cleaning. It was decided to measure the changes in all behaviours to yield records of the change in performance of each different act across starvation.

Change in motivational variables alters the order of prepotency in activities (Hinde, 1970), and Fentress (1968a and b), from a study of two vole species, presented evidence that there was a specific level of arousal which was optimum for grooming. Ollason and Slater (1973), found that in male zebra finches, locomotion and behaviours associated with it were commonest in the morning and declined during the day, whilst behaviours not associated with it showed opposite trends. They suggested that the changes in behaviour could be accommodated in a simple model involving a single (arousal-like) variable showing a 24-hour cycle. It was

hoped that for the tsetse, analysis of the occurrence of all behaviours across starvation in an actograph would reveal any shift in the balance between the behaviours, which might reflect the increased 'hunger'.

Identical observations were also made of all behaviours spontaneously performed in a large arena, the presumption being that any difference between behaviour in the arena and that in the actograph should give an indication of any influence of peripheral factors, such as space, on the performance of a spontaneous burst of activity.

In addition, recordings were made of the response to a moving black stripe immediately after a spontaneous burst of activity, and in the middle of a gap, i.e. period of no activity, between two bursts. From these observations, it was hoped to show whether the response to visual input showed the same changes across a gap between bursts, as does the tendency to perform the next new spontaneous burst. It was hoped that examination of changes across starvation would show whether visual responsiveness and the tendency to perform spontaneous locomotor activity change in close parallel in individual flies across starvation, or in some different manner.

CHAPTER 2

2. GENERAL METHODS

2.1. Standardisation of flies

Whenever possible flies used for experiments were standardised for emergence weights and nutritional background.

Teneral flies were used only if their emergence weights fell close to the mean of the observed distribution of emergence weights.

Mature flies were used only when their emergence weights, the weight of blood imbibed at all three meals per mg fly emergence weight, and the weight of blood imbibed at the third meal only per mg fly emergence weight, were close to the means of the observed ranges of these three measurements.

Details of emergence weights and nutritional history are given in Appendix I.

2.2. Fly Maintenance

Pupae of Glossina morsitans morsitans (Westwood) were received by post from the Tsetse Research Laboratory at Bristol each week. The pupae were collected on a single day. Upon receipt, the pupae were transferred to a cage in a C.T. room maintained at 25 ± 1°C and 65 ± 5% R.H. and with a 12 hour light:12 hour dark cycle.

Flies were collected within 12 hours of emergence, and were weighed on a 50 mg torsion balance whilst under light (no longer than 1 minutes' exposure) carbon dioxide anaesthesia. Flies were then transferred to a 38 mm x 64 mm plastic tubes, with nylon netting at one end. Flies were kept in these tubes at all times unless otherwise stated.

Flies were fed on the ears of lop-eared rabbits by securing the tube, netting end down, on to the rabbits' ear until imbibition had ceased.

Unless otherwise stated, the feeding schedule was as follows. Flies were fed in the morning, the first meal being given once one clear day had passed since emergence. Two further meals were given, each when three clear days had lapsed since the last blood meal. Flies were weighed in their tubes immediately before and after each engorgement, so that the weight of blood taken at each meal could be calculated and used to standardise flies for use in experiments.

Flies which had received three blood meals as described and were 10 days old are referred to as 'mature' flies throughout. Bursell and Kuwenga (1972) showed that in laboratory flies, thoracic muscle development is completed in 8-10 days and after 4 blood meals. Flies which had received no meals and had retained the immature, partly developed cuticle (Hargrove, 1975), and which had only partly developed flight muscles and a characteristic soft feel (Bursell, 1961b) are described as 'teneral' throughout. Unless otherwise stated, all flies used were males.

2.3. Recording of day number

With the exception of the experiments into the probing response, the following procedure for numbering the days of an experiment was always followed.

For teneral flies, the day of emergence was called day 0, the following days day 1, day 2, etc.

For mature flies, the day of the last feed was called day 0, the following days day 1, day 2, etc.

2.4. Extraction of data from Edgecumbe Peebles traces

Brady (1970) made a study of spontaneous activity performed by Glossina morsitans in actographs. From the pen markings on the output from an Edgecumbe Peebles recorder, he showed bursts of intense flight activity to occur, each burst being of ca. 1 minute in duration.

Because of the high variability in numbers of flight bursts per day shown by individual flies (Brady, 1972a), comparative analysis could most easily be made by converting the raw results to percentages. In some cases, activity by each fly across starvation was summed, and then each days activity expressed as a percentage of the total. In other cases, each days activity was expressed as a percentage of the activity on day 1 for each fly. The percentages from all flies were then used to calculate mean activity per day.

CHAPTER 3

3. MEASUREMENT OF SPONTANEOUS LOCOMOTOR ACTIVITY

3.1. Materials

3.1.1. Actographs

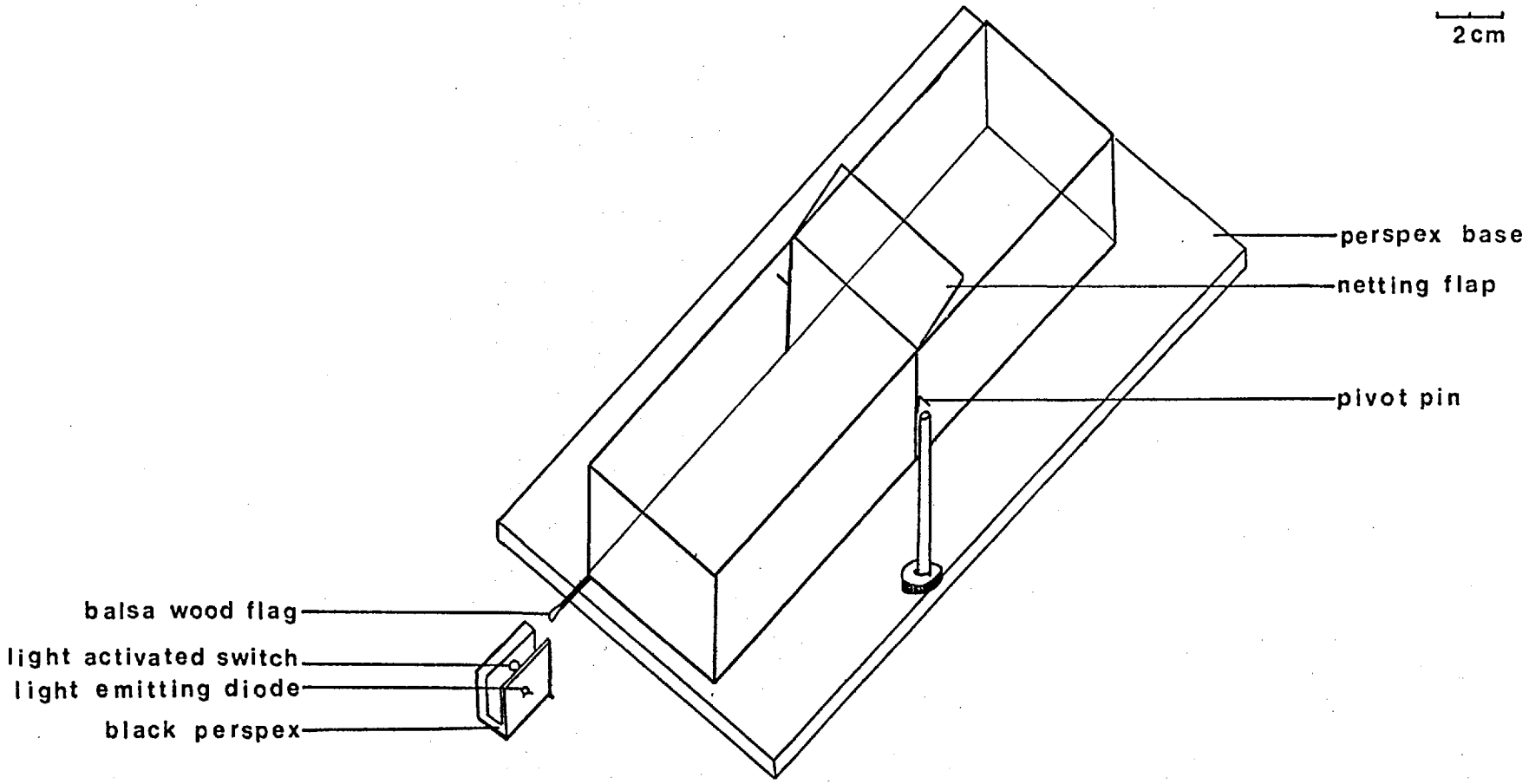
Rocking box actographs were made from 2 mm. square balsa wood strips and nylon netting. Six actographs in each of two different sizes were made. For fully active flies the size was 18 cm. x 5 cm. x 3.2 cm., giving a volume of 288 ml. For flies incapable of flight the size was 9 cm. x 4 cm. x 2.5 cm. with a volume of 90 ml.

The boxes were balanced from pivot pins attached just above the centre of gravity half way along the side of the box. Two 5 cm. countersunk screws were fastened by nuts through holes drilled in a clear perspex base, so that the screws stood each side of an actograph. A groove drawn across the flat top of each screw acted as a mount for the pivot. This arrangement ensured that the weight of any fly resting away from the centre would tip the actograph.

From one corner at the front of each actograph projected a 1.3 cm. length of entomological pin bearing a 1 mm. x 2 mm. x 0.5 mm. balsa wood flag. Flies could be posted into the boxes through overlapping netting flaps in the top, and the flaps fastened with cotton thread.

During experiments the actographs were enclosed in a 26 cm. x 12.1 cm. x 10.2 cm. box with sides made of 1.3 cm. chipboard, painted white. The base was of 3 ply wood and painted black. In the front left hand corner of the box a 20 mm. x 9 mm. x 15 mm. block of black perspex was fixed. The perspex had a groove of 4 mm. width milled in the upper 2/3, leaving 1 mm. of perspex width on one side into which was embedded a light emitting diode, and leaving 4 mm. width perspex wall the other side into which was embedded a light activated switch. The actographs were arranged inside the chipboard box so

FIG. 3.1. A ROCKING BOX ACTOGRAPH.



that when the actograph rocked, the balsa wood flag would pass between the light emitting diode and the light activated switch. An actograph is illustrated in Fig. 3.1.

The lid of each box was of 5 mm. opal perspex which had 4 holes drilled to accommodate screws embedded in the wall of the box. The lid was fastened down by wing clips.

The rear end of each box had a circular pattern of small holes drilled through. These holes were surrounded by a 6 cm. diameter white plastic filter funnel glued broad end to the box outside. Odour free air was supplied through the filter funnel as follows. A 7.5 cm. squirrel cage fan onto which had been attached an 8 cm. x 14.3 cm. x 8.8 cm. plywood air pressure chamber passed air into a 2 cm. diameter rubber tube, and then into a sealed 23 cm. x 11.5 cm. x 8.2 cm. chamber of activated charcoal. From this chamber 1.3 cm. tubes carried the odour-free air into the tube end of each filter funnel.

The actographs were illuminated by 2 40 watt Atlas daylight fluorescent lights operated by a time clock. These were suspended at a height above the boxes which gave a 1200 lux reading inside the chipboard box.

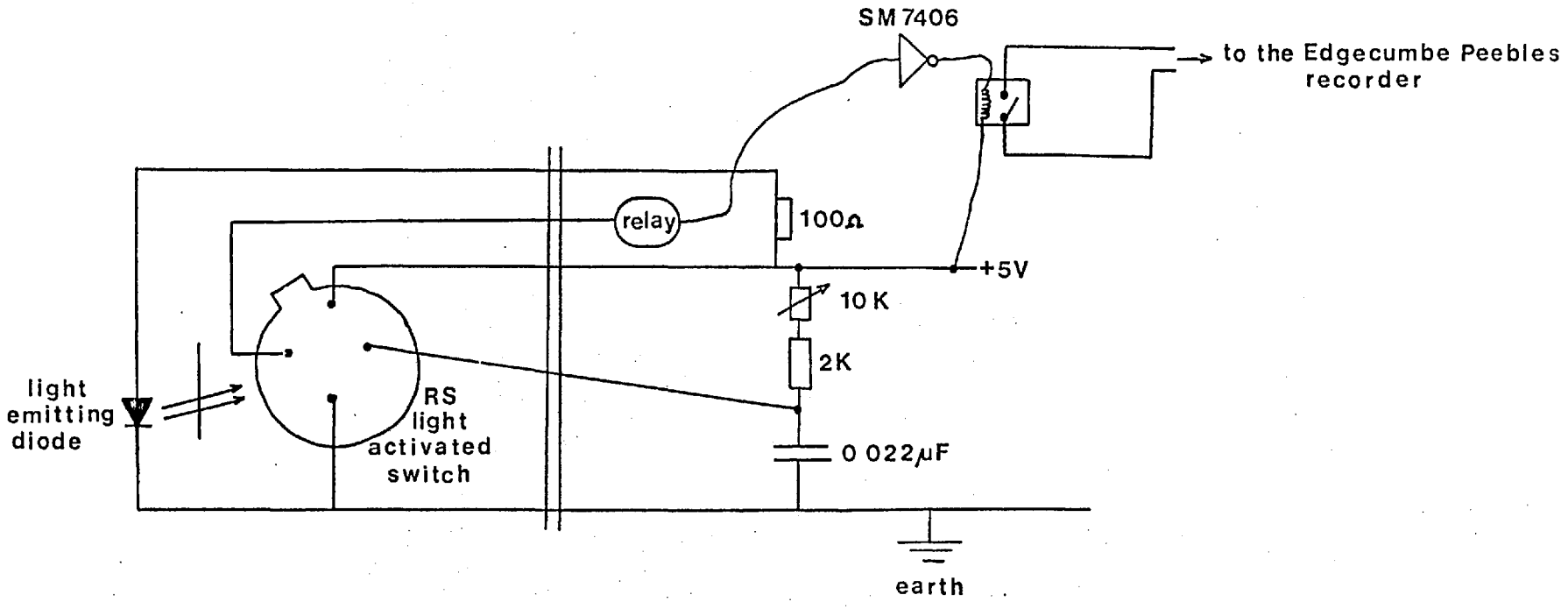
3.1.2. Recording system.

Fig.3.2. shows the circuit taking information from the light activated switches to the recording machine. A 5 volt power supply and 6 circuits were built. The path of each circuit can be traced from Fig.1. The small voltage changes from the light activated switches were converted by the reed relays into the on/off impulses needed to stimulate an Edgecumbe Peebles 6 channel pen event recorder.

3.2. Method

Mature flies were pooted singly into the actographs the evening after the third bloodmeal, and the pen recorder switched on, so that records were available from lights on the first day after the third feed, hereafter called day 1, until recording was terminated.

FIG.3.2. THE CIRCUIT TO RECORD THE OUTPUT FROM THE LIGHT ACTIVATED SWITCHES



Teneral flies were pooted singly into the actographs the evening of the day of their emergence, and recording begun, so that records were available from lights-on the following day, called day 1 hereafter.

Actographs had previously been used successfully to measure spontaneous locomotor activity of blowflies (Green, 1964, a) and of tsetse flies (Brady, 1970). Brady (1970, 1972a, 1975) had shown that the mean duration of flight bursts did not change significantly across starvation, and that the number of flight bursts per day is directly proportional to the amount of time spent in flight per day. This was confirmed for 15 mature flies with recordings made across starvation with paper from the Edgecumbe Peebles recorder emerging at 152 mm./hour. For these flies, the mean length of flight burst across starvation was calculated, and data are given in table 3.1.

Table 3.1. Mean length of flight burst in seconds. ± S.E.

DAYS SINCE LAST FED				
1	2	3	4	5
44.80	45.02	43.66	41.93	46.23
±2.35	±2.48	±2.71	±2.59	±2.44

These lengths were calculated from fine measurements of the width of pen markings from each of which was subtracted ½ pen width to allow for ink spread, and with any gap longer than 30 seconds taken to indicate that the ongoing burst had ended. Since the flight lengths do not change across starvation, any data extracted from such actograph pen recordings are given as flight bursts per day throughout. (In Chapter 9 only, where observation allowed recording of other behaviours in addition to flight, the term 'activity burst' is used).

Six teneral flies were also measured for spontaneous locomotor activity across starvation, with the paper from the Edgecumbe Peebles emerging at 152 mm./hour. Both these, and the mature flies provided control measurements of change in spontaneous locomotor activity across starvation in untreated flies. All other recordings were

made with the paper emerging at 2.53 cm./hour.

Unless stated otherwise, only the number of flight bursts during lights-on were extracted from the Edgecumbe Peebles output. Brady (1972 a) had found that mature flies in a 12:12 hour light/dark regime exhibited only 0.012% per hour of the total daily activity during lights out over the first four days of starvation. The lights-out artifact, a burst of activity following the sudden onset of darkness was also always excluded.

3.3. Results

The results from the 15 mature, and 6 teneral flies measured for change in spontaneous locomotor activity across starvation were broken down into activity per hour during the 12 hours of the photophase. The percentage of the total activity over all 5 days which occurred in each hour was calculated for each group. The results are given in Table 3.2. These results are given graphically in Figs. 3.3. and 3.4. from which it can be seen that for mature males there

Table 3.2. Mean percentages of total activity for all days to occur in each hour.

		Hours since lights on											
Flies	Day	1	2	3	4	5	6	7	8	9	10	11	12
Mature N=15	1	4.8	3.3	1.8	0.7	0.4	-	-	0.2	0.3	0.7	0.5	0.5
	2	5.7	3.2	2.2	0.3	0.3	0.2	-	-	0.1	0.3	0.4	1.3
	3	9.5	4.5	2.2	0.4	0.2	0.7	-	0.1	0.3	0.4	0.6	1.3
	4	9.8	5.3	1.9	0.6	0.3	0.2	-	0.3	0.3	0.8	0.7	3.0
	5	12.3	6.5	2.9	1.3	0.9	0.3	0.2	0.4	0.5	0.9	0.9	2.3
Teneral N=6.	1	8.8	3.4	1.7	0.3	-	-	-	0.3	-	0.1	0.3	0.6
	2	14.2	6.8	4.0	0.3	0.6	-	1.2	1.4	0.6	1.2	2.0	2.3
	3	15.3	6.3	3.0	1.3	1.2	-	-	1.2	-	0.6	0.8	0.8
	4	7.1	3.7	0.8	0.1	0.6	-	-	-	-	-	0.8	3.0
	5	1.4	0.6	0.3	0.6	0.1	0.6	-	-	-	-	-	0.6

is a linear increase in activity up to day 5. For teneral males, the activity increases up to day 3, and is then followed by a pre-death decline.

FIG.3.3. CHANGE IN SPONTANEOUS LOCOMOTOR ACTIVITY WITH STARVATION OF MATURE MALES N:15

Curves smoothed by three point sliding means. Data from periods of lights-on only.

Left hand ordinate: hourly activity as percentage of total all days (∘). Right hand ordinate: daily activity as percentage of total all days (▲).

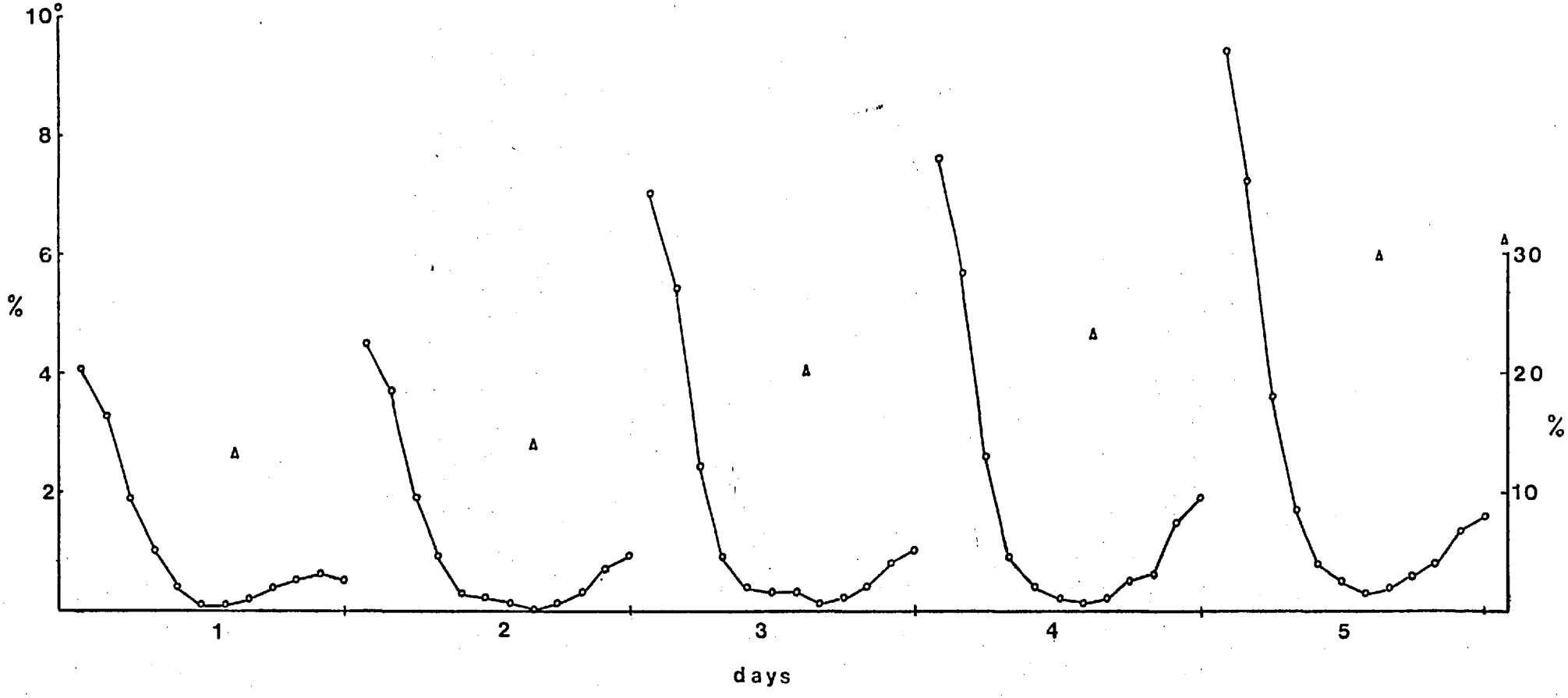
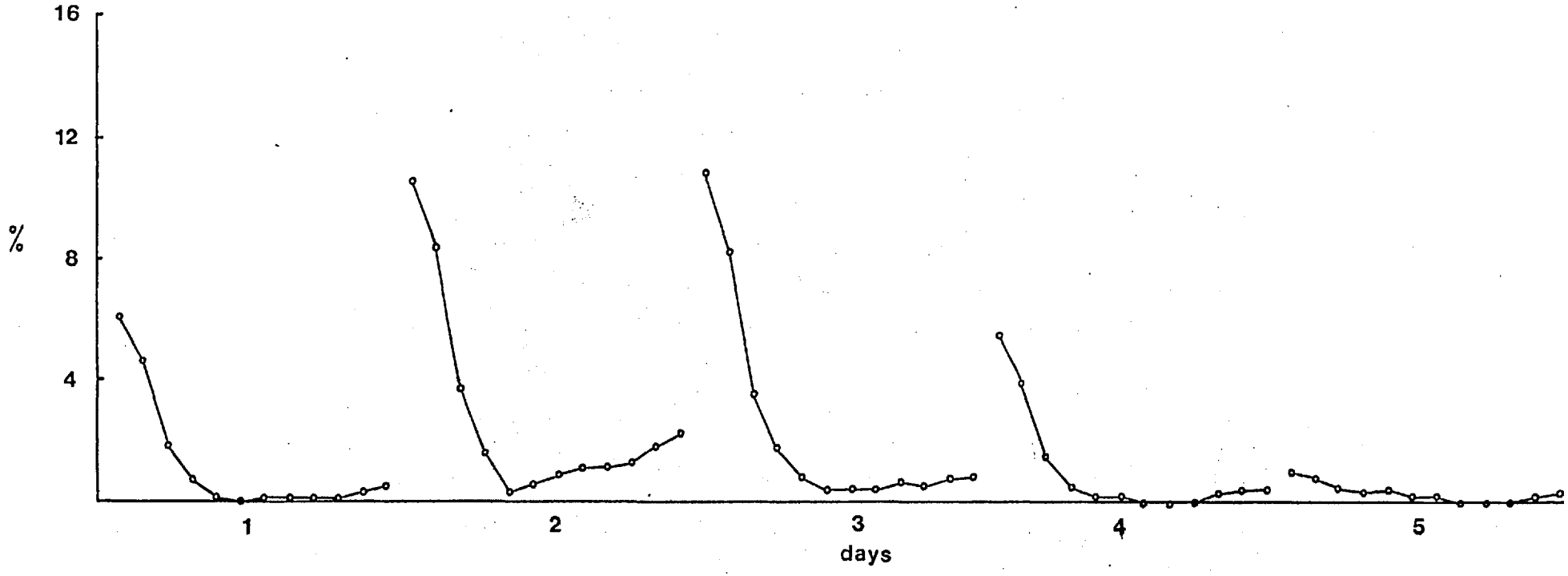


FIG. 3.4. CHANGE IN SPONTANEOUS LOCOMOTOR ACTIVITY ACROSS STARVATION IN TENERAL FLIES.

Ordinate: hourly activity each day as percentage of total activity – data from periods of lights-on only

Curves smoothed with three point sliding means



3.4. Discussion

These results show that in the actographs under discussion, both mature and teneral flies show similar circadian rhythmn and similar increase in activity with starvation as was reported by Brady (1972 a).

CHAPTER 4

4. INVESTIGATION OF THE ROLE OF WEIGHT IN THE CONTROL OF SPONTANEOUS LOCOMOTOR ACTIVITY

4.1. Wing clipping

4.1.1. Method

Wing clipping was performed at two different times on day 3, neither time being completely satisfactory. Wing clipping immediately after lights-out on day 3 had the advantage of not interfering with the activity record for day 3, but had the disadvantage of adding approximately 40 minutes to the light regime for that day. Wing clipping during the period of the day with the lowest level of activity, i.e. some 7 hours after lights-on, had the advantage of not adding to the days' length, but meant that 40 minutes of recording time were lost, and also, that the afternoon's record of activity, strictly post treatment, was added to the pre-treatment record for the morning to give the reading for day 3. However, since the evening peak of activity was found to be only 22% of the days' total, the addition of any change in this to the more substantial reading of the morning's activity for day 3 was not felt to be prohibitive.

Two different ways of anaesthetising the flies were tried. Firstly flies were transferred from the actographs to the usual storage tubes. The tubes were placed, plastic lid end down, onto a bed of chip ice. It was found that two minutes was long enough to anaesthetise the flies and to enable clipping of the wings in an exact position. In a second method, in an attempt to reduce the effect of the cold in depressing subsequent activity, carbon dioxide anaesthetic was used, using only just enough gas to immobilise the flies. Moloo and Kutuza (1975) found that toxicity and bloating of the abdomen increased with increased exposure, but were low at 10 minute exposures, so that the 1 minute used here should have had negligible effects. It remains, however, a factor whose effects are unknown.

After treatment with anaesthetic, flies' wings were clipped by cutting in a plane parallel to the longitudinal axis of the body, with the wing extended, the cut passing through the junction between vein iv and the posterior cross vein at e. This removed ca. 33% of the leading edge of the wing, and ca. 27.5% of the total area of the wing. Cutting in this position would not have removed, but may have increased the load on, sensory cells in the remigial cell of the wing. Newstead, Evans and Potts (1924) considered that these cells may be mechanoreceptors. Control flies were anaesthetised only and handled, but were not wing clipped.

After treatment, flies were returned to the actographs, and spontaneous locomotor activity monitored to day 7 or 8.

4.1.2. Results

The results are given as flight bursts per day, and then each day's activity is expressed as a percentage of the activity on day 1, referred to hereafter as percentage activity. The results for mature males and mature females are treated separately since the female must have a more complex weight measurement mechanism than the males due to her viviparity.

Flies anaesthetised by freezing, and wing clipped after lights out are placed in category a; flies anaesthetised by freezing and wing clipped at time of lowest activity during the day are placed in category b; and flies anesthetised by CO_2 during time of lowest activity in c. For the analysis, amalgamation of all data for mature males is labelled d.

For each category of mature males, and for the mature females, the mean daily percentage activity $\pm$ S.E. is shown in Tables 4.1. and 4.2. respectively, and Figs 4.1. and 4.2.

Table 4.1. Mean percentage activity per day mature males ± S.E.

Category and Treatment

	a		b		c		d	
DAY	Clip	Control	Clip	Control	Clip	Control	Clip	Control
2	162.2±24.1	175.6±24.8	148.6±13.2	115.4± 8.8	124.1±12.8	105.4±14.6	147.38±11.9	139.7±13.3
3	181.1±18.0	185.5±25.9	165.6±13.8	128.0±21.4	164.4±18.6	159.8±23.3	172.1 ±10.1	163.8±14.9
4	160.4±16.5	172.8±24.6	119.8±18.2	96.1± 9.4	146.2±25.9	113.6±?2.4	145.5 ±11.6	136.3±14.3
5	161.5±17.5	245.7±50.8	145.2±34.5	74.9±23.1	202.3±44.5	177.8±24.5	169.2 ±17.5	183.7±27.1
6	145.9±17.5	247.4±46.7	127.3±32.6	114.0±24.7	173.2±48.7	148.2±39.1	148.8 ±20.0	193.7±29.2

FIG. 4.1. THE EFFECT OF WING CLIPPING ON ACTIVITY OF MATURE MALES

Ordinate: Flight bursts per day as a percentage of the bursts on day 1.

o, control flies ◻, wing clipped flies ↓, time of treatment. See text for a, b, c, d.

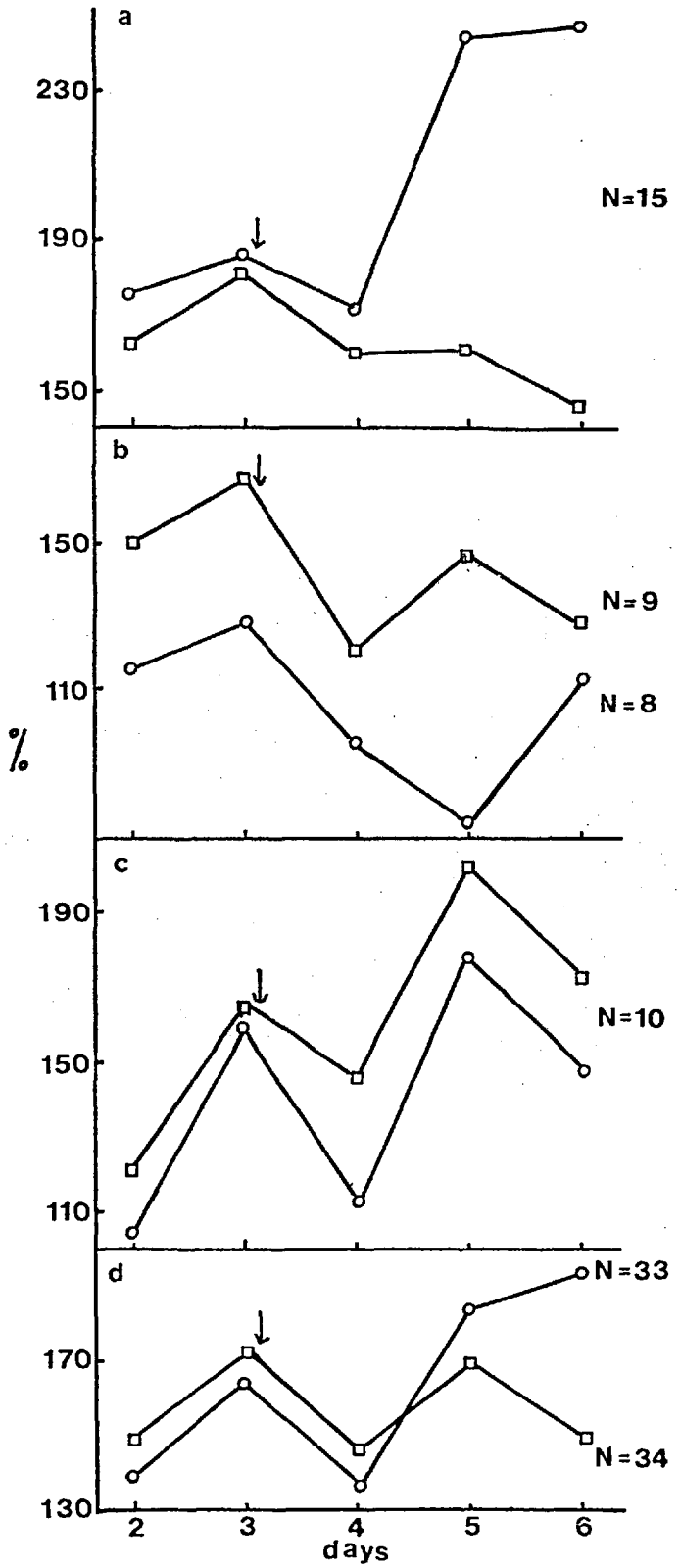


Table 4.2. Mean percentage activity per day mature females ± S.E.

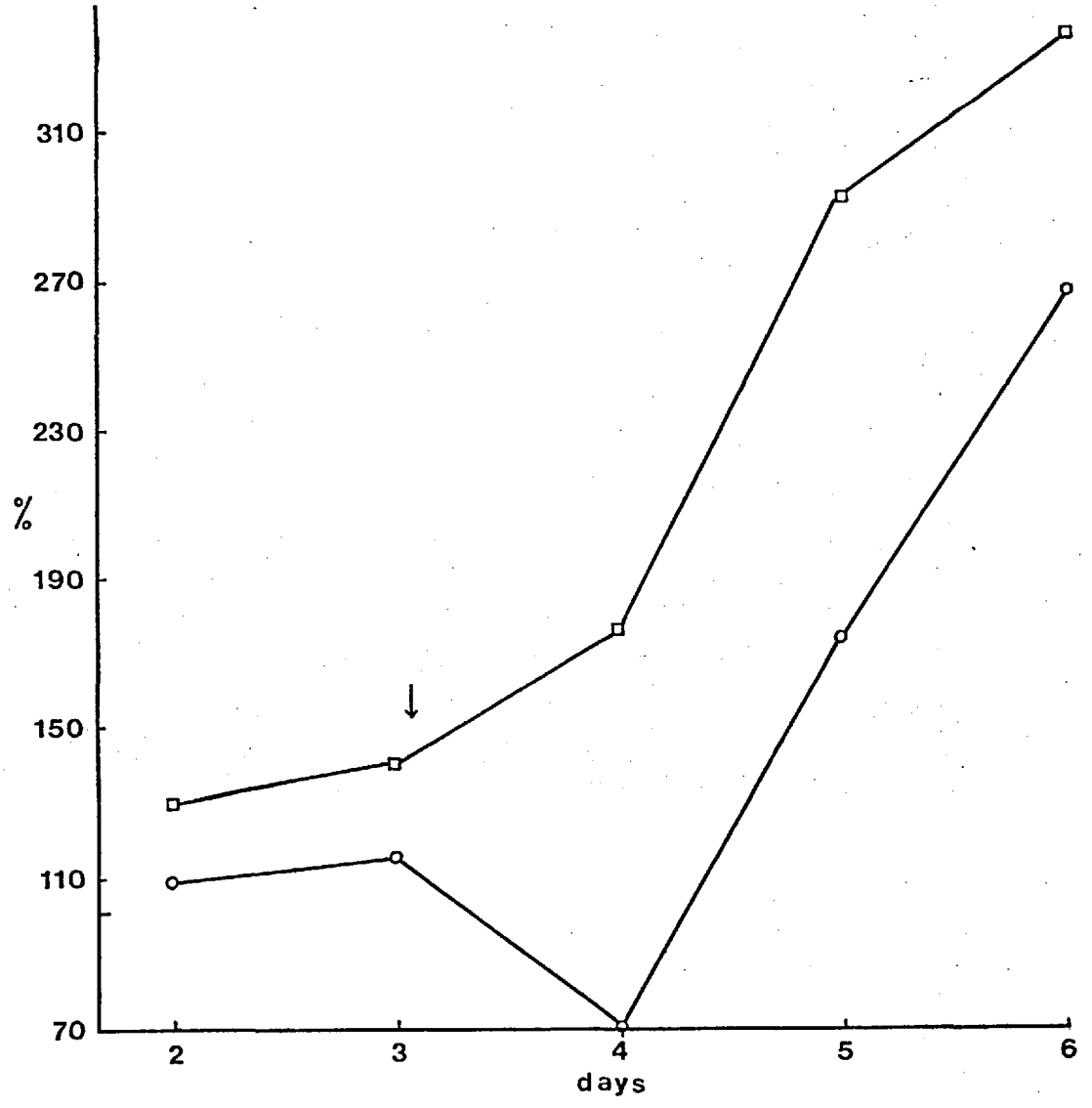
Category and Treatment

	C	
DAY	Clip	Control
2	131.4± 25.9	109.0± 12.6
3	141.0± 17.9	115.2± 12.1
4	174.7± 53.9	71.0± 13.13
5	293.4±100.0	174.0± 50.1
6	336.6±120.4	268.0±129.4

FIG. 4.2. EFFECT OF WING CLIPPING ON THE NUMBER OF FLIGHT BURSTS PER DAY. MATURE FEMALES

Ordinate: flight bursts per day as percentage of bursts on day 1.

o, control flies ▫, wing clipped flies ↓, treatment time, type c. N=9



These results were tested with 'Student's' t for any difference between the two means of percentage activity of treated and control groups. Day 3 before treatment was tested to establish that there was no difference between control and treated groups before treatment, and day 4 was tested for difference after treatment. For the mature males, group a day 5 viewed from Fig.4.1. showed a large difference between control and treated flies, and so this was also tested. However, in this case, as also in the mature females on day 4, there was a significant difference between the variances of the two groups ($F = 8.43$, 14 x 14 df; $F = 16.85$, 8 x 8 df, respectively) and so the t test for these two pairs was performed as described by Bailey (1959) for such a set of values.

The results of the t tests are given in Table 4.3., from which it

Table 4.3. Results of t tests for difference between mean percentage activity treated and control flies

		Group and category				
		Mature males				Mature females
DAY		a	b	c	d	c
3	df	28	15	18	65	16
	t	-0.14	-1.51	0.15	-0.46	1.19
	p	>0.1	>0.1	>0.1	>0.1	>0.1
4	df	28	15	18	65	9
	t	-0.42	-1.11	0.95	-0.50	0.03
	p	>0.1	>0.1	>0.1	>0.1	>0.1
5	df	17				
	t	1.57				
	p	>0.1				

can be seen that there is no significant difference between control and treated flies on any day.

4.1.3. Discussion

The results show that the high correlation found by Brady (1975) between activity level and weight does not depend upon measurement of weight by the wings, and that the remigial pores on the remigium of the wing, thought by Newstead, Evans and Potts (1924) to be mechanoreceptors, are probably not involved in weight measurement.

That weight is not monitored by the wings is perhaps to be expected for three reasons. Firstly, if weight was measured by the wings, the weight-associated change in threshold for spontaneous locomotor activity would depend upon inputs available for less than 1% of the time, since the mean daily time spent in activity in actographs is only 12.5 minutes (Brady, 1972a). Short of some form of memory system operating of weight at last flight, it is easier to envisage threshold being set by some continuously monitored variable such as weight on legs.

Secondly, when examining why flight occurred, if the causal factor for the drop in threshold to allow initial take-off were due to loss in weight measured by the wings, then that input would not be available until after take-off.

Thirdly, considerable fraying of wings occurs in the wild. Whilst wing mutilation causes an increase in wing beat frequency (Hargrove, 1975a), and the apparent increased load resulting from a reduced wing span is responded to in this way, it would not be advantageous to flies to perform fewer flight bursts due to an apparent increase of weight caused by natural fraying of the wings.

4.2. Leg Loading

4.2.1. Materials

A series of polystyrene balls (diameter of 12.5mm) was made. Different weights were achieved by inserting varying amounts of lead shot into each ball. For heavier weights the ball was halved, the core removed to allow space for the volume of lead, and the

two halves glued back together with Durofix. Ten balls were made to each of the following weights: 100, 150, 200, 250, 300, 350, 450, 550mg. Three further balls of 650, 750 and 850mg were made to test mature females.

4.2.2. Method

Flies being tested were suspended by matchsticks or by string mounts from a strip of wood held by 2 clamps horizontally above the bench at a height of 23cm. The flies were protected from any visual stimulus change by cardboard partitions attached to a 30cm high cardboard rear wall and were mounted 'eyes away' from the observer. The ball being tested was offered to the suspended fly from the observer's hand, and thus no control of olfactory input was made, but this input should have been similar on each day. Just after 'accepting' a ball flies often began to walk, which sometimes resulted in a drop due to 'missed footing'. Therefore, if a fly dropped a ball within 30 seconds of being offered it, the ball was re-offered. A second drop, or a first drop occurring after 30 seconds, but before 3 minutes had passed since holding began, was viewed as a positive rejection, and scored as a drop. Sometimes when very hungry flies were offered heavy balls, the weight would stretch the legs downwards, resulting in the ball being only incidentally suspended from the tarsal claws. This was also scored as a drop, since adequate muscular effort was not being made to hold the ball up.

Preliminary tests had shown that for mature males and both sexes of tenerals, a range of ball weights from 100-550mg allowed newly fed or emerged flies to hold nearly all balls, whilst very hungry flies would drop all but the lightest balls. For mature females, it was necessary to use an extended range of 100-850mg to attempt to achieve a similar gradation of drops.

Unless stated otherwise, three readings a day were taken, one each during the morning and evening peaks of locomotor activity, and one during the midday trough. Immediately before each reading, flies were offered a 100mg ball to hold onto for one minute. This was an important preliminary step to testing, since flies permanently mounted on matchsticks, or flies mounted just prior to testing held their legs in an unnatural 'curled-under' position. After this, a minute was allowed to elapse before testing began. Flies were offered balls in ascending order of weight. When the order was reversed for some trials, there was no difference in the number of drops recorded at each weight. When one weight had been tested, a minute was allowed to elapse before flies were offered another ball. (For flies which had dropped their balls early in the test period, some few minutes may have elapsed before they were offered another ball, but short of testing each fly individually, this could not be avoided).

Flies which were mounted permanently had a matchstick attached to their dorsal thorax by beeswax, just after the third meal. This mounting medium was used to avoid toxicity from synthetic products and because its low melting point enabled it to be used without causing thermal damage to the fly. The matchsticks were suspended from plasticene anchorage points on the horizontal wooden strip.

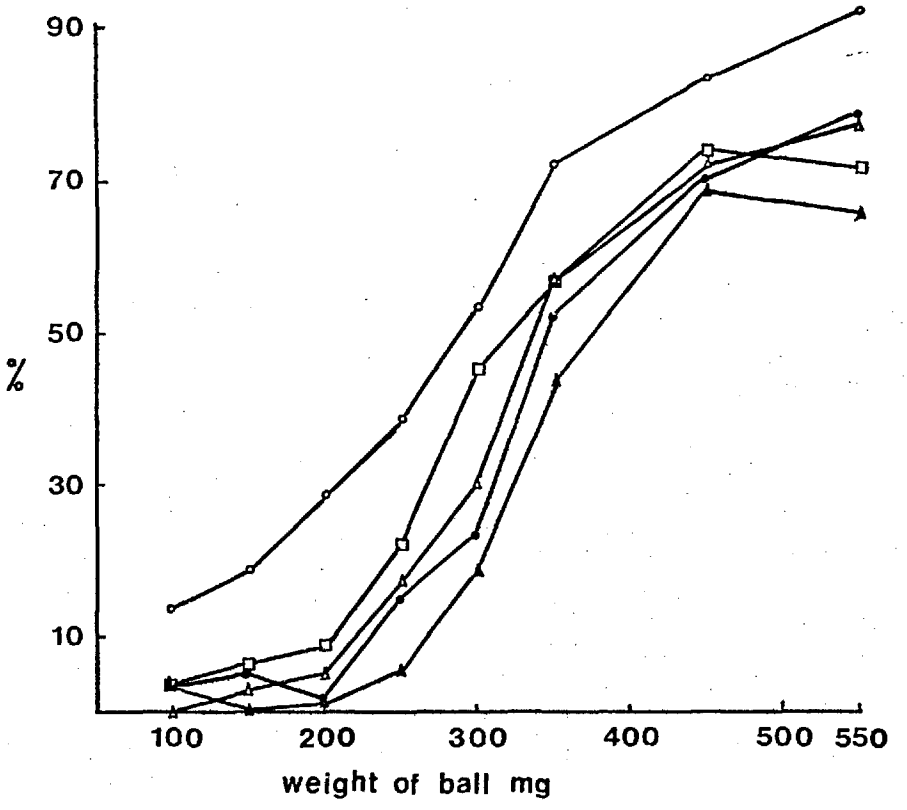
Flies which were mounted for the duration of each test only were treated as follows. Just prior to the third meal, flies were held gently in the author's hand so that the dorsal thorax just made contact with a small amount of beeswax on the end of a 9mm piece of string suspended from a clip. Application of a hot pin to the wax caused it to melt, and a small volume to flow over the dorsal thorax. The fly was then returned free to the normal storage tube, and fed as usual. In this way, the weight of blood imbibed at that third meal, and the subsequent weight loss would have been recorded by any monitoring system as change in fly weight inclusive of the weight of the string and the wax.

Thus, with the exception of the duration of testing, string-mounted flies always had access to information about their own weight. Immediately prior to testing, flies were removed from the storage tubes, and the free end of each string was gripped by one of a series of small bulldog clips which had been nailed to the horizontal wooden strip. Flies were left suspended during one testing period, and then were returned to the storage tubes until the next period.

Preliminary tests were made on both sexes of permanently mounted teneral and mature flies. To investigate more fully the change in weight sensitivity of the legs with starvation, and in an effort to investigate the impact of permanent mounting and feedback from a fly's own weight on weight-sensitivity of the legs, the following was tried. 29 mature males mounted permanently, and 20 which were string-mounted were tested for change in weight sensitivity of the legs across starvation.

To investigate if the observed changes were reversible by food intake, permanently-mounted teneral males and females, and mature males were each fed early in the morning, of the first day of post-feed testing, by the observer holding the matchstick at an appropriate position over the ear of a lop-eared rabbit. Flies fed this way did feed willingly, but meal sizes were observed to be much smaller than under normal circumstances. One batch of string-mounted flies was tested across starvation, and then fed in the normal way in the storage tubes, and then tested again. This group of flies was fed varying weights of bloodmeal, and the weights noted so that a range suitable for testing for any correlation of bloodmeal size to degree of change in the tendency to drop balls was available.

FIG.4.3. PERCENTAGE OF STRING-MOUNTED MATURE MALE FLIES DROPPING BALLS OF DIFFERENT WEIGHT. N=20



4.2.3. Analysis of Results

Fig.4.3. shows the form of the change in the percentage of flies dropping balls of different weights. This is a quantal, all or none, response, and there was an increase in the number of drops with an increase in the weight of the ball. The curves are sigmoid because they are cumulative, since when small weights were dropped, large weights generally were also. The slope of most of the curves is steepest around the 50% response level, and this level thus gives the most accurate way of measuring any change in response of different groups of flies across starvation.

The data for five days of starvation from preliminary batches of flies, and from 29 permanently mounted and 20 string mounted mature male flies were converted to 50% drop estimates for each day by probit plane analysis (Finney, 1971), using the S102 program stored on the University of London's CDC 600 computer. This analysis reduces the asymmetry of the curves by transforming the weight logarithmically, and then transforms the sigmoid curve to a straight probit line, from which the 50% response is derived.

4.2.4. Results

The weights at which 50% of flies dropped balls on different days in the preliminary investigation are given in Table 4.4. and Fig.4.4., and in all groups there was a decline in the weight of 50% drop across starvation, from similar drops on day 1 for all groups. A batch of mature virgin females tested with weights 100-850mg did not show a progressive change in 50% drop across starvation, and held weights 2-3 times heavier than the other groups with 50% drop on day 3 being at the greatest weight of 1333mg predicted from the analysis.

FIG.4.4.WEIGHT AT WHICH 50% OF FLIES DROPPED BALLS ACROSS STARVATION

∘ , teneral ♀ . ▵ , teneral ♂ . • , mature ♂ .

Bars = 95% confidence limits

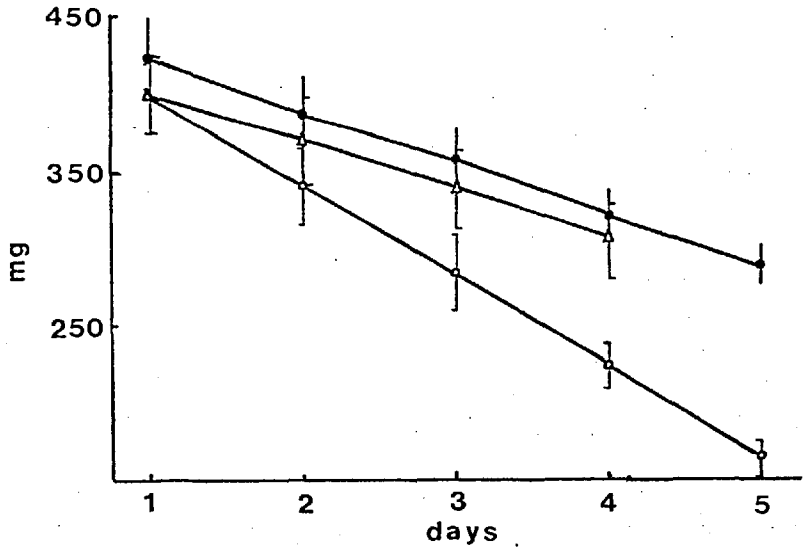


Table 4.4. Weight in mg at which 50% of flies dropped balls ± S.E.

Day: since fed (mature), or emergence (teneral)	Teneral ♀ N = 7	Teneral ♂ N = 6	Mature ♂ N = 10
1	401 ± 28	403 ± 29	425 ± 27
2	343 ± 24	372 ± 28	392 ± 24
3	284 ± 20	341 ± 27	359 ± 21
4	225 ± 15	310 ± 26	326 ± 17
5	166 ± 11		293 ± 14

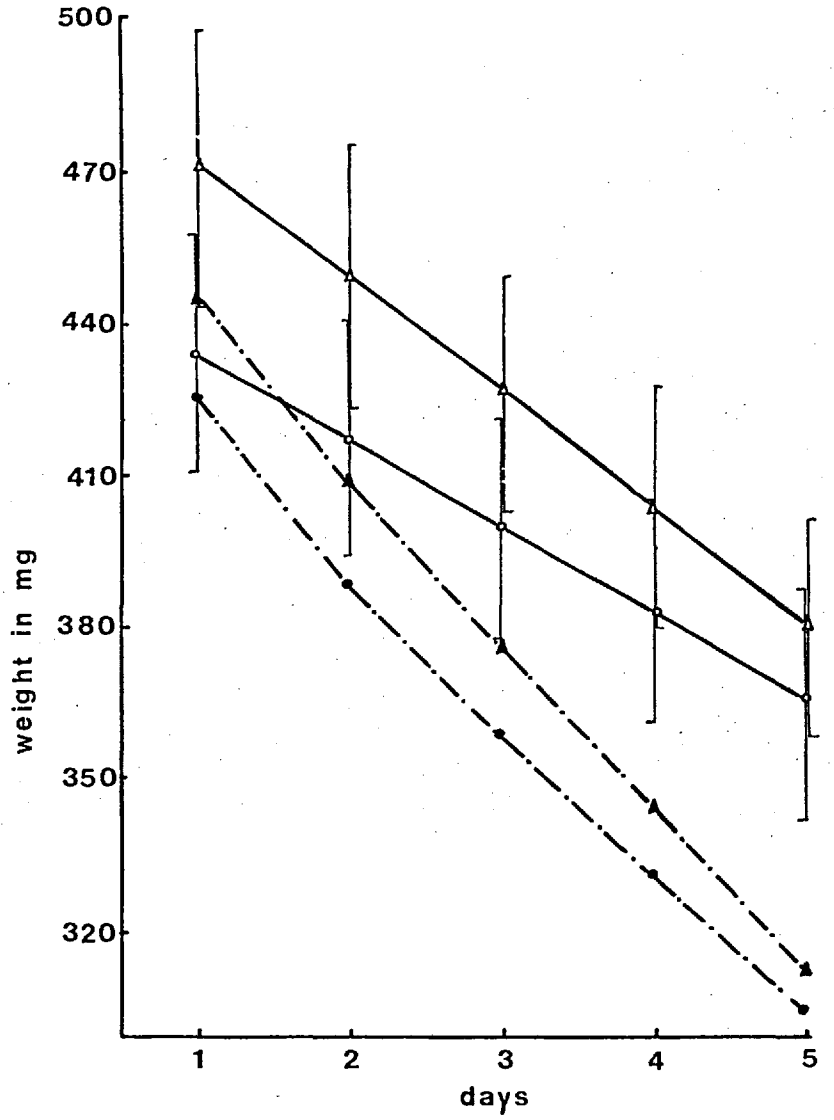
The results of the permanently- and string- mounted mature males are given in Table 4.5. and Fig.4.5. For both of these larger groups of flies, the probit plane analysis indicated that there was a significant interaction, i.e. a synergistic effect, between increased time since the last meal and increased weight of the ball. The analysis gave 50% estimates both from describing the data by a flat plane, and by fitting equations taking account of the interaction to give a better fit to the data. Both estimates are given.

Table 4.5. Weights in mg at which 50% of flies dropped balls ± S.E.

Day	Permanently-mounted flies N=29		String-mounted flies N=20	
	Without interaction	With interaction	Without interaction	With interaction
1	472 ± 27	443	434 ± 23	424
2	449 ± 26	407	417 ± 23	388
3	427 ± 25	375	400 ± 22	358
4	404 ± 24	344	383 ± 22	331
5	381 ± 22	312	366 ± 21	307

FIG.4.5. WEIGHTS AT WHICH 50% OF FLIES MOUNTED BY TWO DIFFERENT MECHANISMS DROPPED BALLS ACROSS STARVATION

Permanently-mounted flies: △, without and ▲, with interaction
String-mounted flies: ○, without and ●, with interaction
Bars= 95% confidence limits



As in the preliminary investigation, flies reached 50% drop at progressively lighter weights as starvation increased. Permanently-mounted flies seemed to hold heavier weights than string-mounted flies, but the difference was not significant. (When the means of numbers of drops as a percentage of the possible number of drops at each weight each day for the two groups were tested they gave $\underline{t} = 0.11$, at 78df with $p > 0.9$). The rate of change across starvation was closely similar for the two groups, and thus the trend in permanently-mounted flies was not due merely to effects of being mounted, nor is it affected by lack of feedback via the legs as to the flies own weight.

The results of the accumulated 25 days of recordings from nine permanently mounted mature males, each tested across a number of days were analysed for any evidence of circadian rhythmicity of the tendency to drop balls. The mean numbers of drops observed at each reading per day were 29.7, 30.6, and 29.6 drops at the morning, noon and evening readings respectively. ($\underline{t}$ tests showed no significant differences). Thus the dropping response is not modulated by a circadian rhythm.

The results of the effect of feeding on the dropping response are shown in Table 4.6. and Fig.4.6. The probit plane analysis could

Table 4.6. Drops per day as percentage of total possible drops. ↓indicates time of meal

Type and Number of flies	Days 1	2	3	4	5	6	7	8	9
Teneral ♀ N=7	29	30	55	65	75↓	64	64		
Teneral ♂ N=6	22	29	42	52↓	46	52			
Mature ♂ N=10	28	29	32	43	48↓	38	51	52	69
Mature ♂ N=6	16	22	19	20	31	44↓	28	30	44

not be used across the change in circumstance as a result of the feed, and so the data are given as drops per day as a percentage of the total possible number of drops that day. All types of fly tested showed a reduction in the percentage number of drops for two days after a meal, before the number of drops increases

again with renewed starvation. A t test between the number of drops as a percentage of the total number of possible drops on the day before the meal and the day after for each of the 29 fed flies showed a significant decrease in the tendency to drop balls ($t = 2.88$, at 56 df with $p < 0.01$). Thus the tendency to drop balls is reversible by feeding. The level of drops does not revert to that on day 1 because the meals taken by mounted flies were small. In the case of the permanently mounted flies, these results from feeding show again that the change in the tendency to drop balls with starvation is a reflection of some aspect of nutritional state, and is not merely caused by duration of mounting.

The bloodmeal intake at the fourth meal of string-mounted flies was corrected for mg/mg emergence weight, and then a regression analysis was performed between the intake and the resultant percentage change in number of drops for each fly. For the first and second days after the meal, the results were not significant, with $p > 0.1$ for both days. However, if the number of drops on the first and second days are averaged for each fly, and then expressed as percentage change, the correlation with blood intake is significant, ($r = 0.55$, $t = 2.46$ at 15 df and $p < 0.05$). The regression is shown in Fig.4.7. Evidently, there therefore is a weak positive association between reduction in number of drops and the amount of blood imbibed.

4.2.5. Discussion

This response is a 'noisy' one, with drops being caused not only by positive rejections, but also presumably by missed footing due to fast walking, and, for lighter weights, possibly also attempts to flex the legs to bring the ball into a suitable position for probing. Nevertheless, the results do show that tsetse flies are sensitive to the weight supported by their legs. The weight at which 50% of flies drop balls declines linearly with starvation in all types of fly tested except mature virgin females.

FIG.4.6. THE EFFECT OF A MEAL ON THE DROPS PER DAY AS A PERCENTAGE OF THE TOTAL POSSIBLE DROPS

↓, time of meal. ○, teneral ♀. △, teneral ♂. ●, and ▲, mature ♂

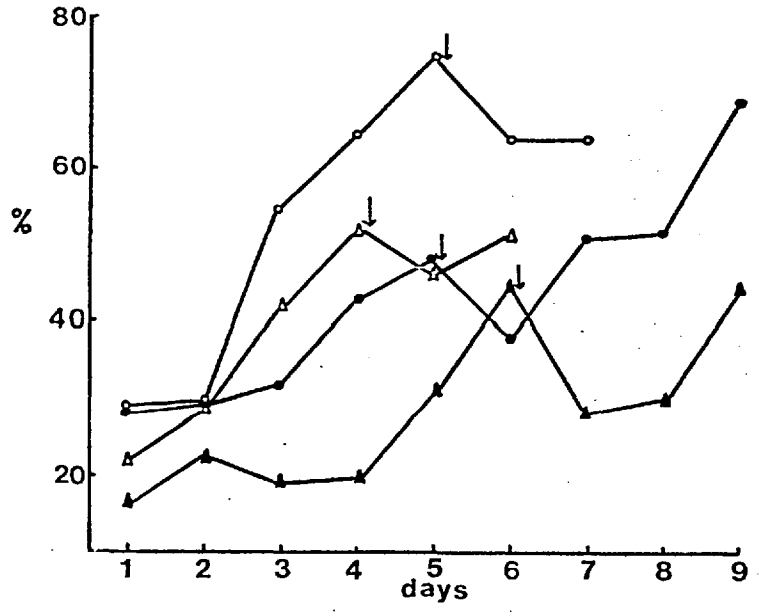
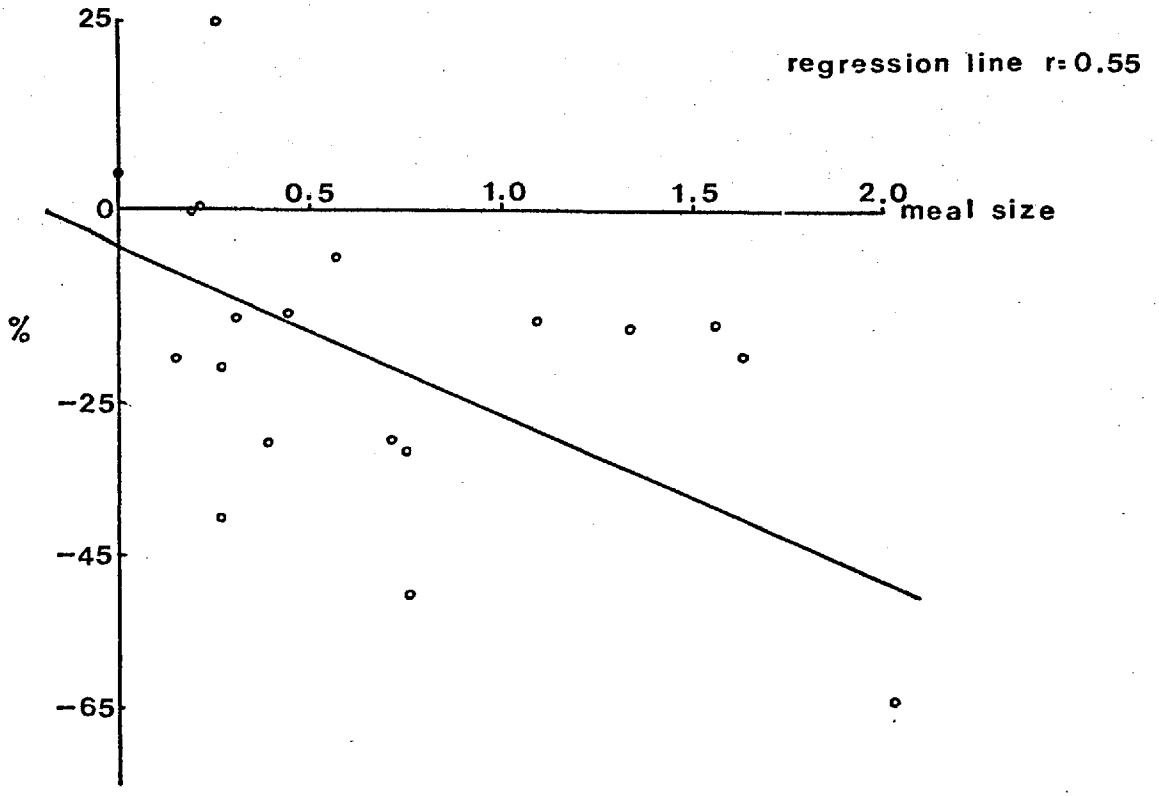


FIG.4.7. THE RELATIONSHIP BETWEEN BLOODMEAL SIZE (mg/mg EMERGENCE WEIGHT) AND MEAN PERCENTAGE CHANGE IN THE NUMBER OF DROPS ON DAYS 1 AND 2 AFTER FEEDING



The large estimated weights of 50% drop for these females may reflect the weight a pregnant female would 'expect' to carry, although these females had not been mated, and teneral females showed the same changes as both groups of males.

The increase in the number of drops with starvation can be reversed by feeding. Thus sensitivity to weight of the legs is a plausible mechanism whereby changes in whole fly weight across starvation may be monitored and relayed to the CNS, resulting in the starvation-induced changes in behaviour. However, permanently-mounted flies, with no feedback from their legs, as to their real weight show the same 50% drop changes with starvation and with a meal, as do string-mounted flies. Whilst these two observations do not conflict with the suggestion that in a free fly the weight sensitivity of the legs may relay information to the CNS about the state of starvation, they do raise the question as to how, in this experimental context, the change in weight sensitivity of legs of permanently mounted flies is modulated. The duration of mounting is ruled out (above), and so perhaps abdominal stretch as a result of the meal may play a part in influencing the tendency to support weight by the legs. That the weight flies will hold in their legs may truly reflect the weight the fly would 'expect' the legs to bear, for example when hanging on a tree-trunk, is indicated by the significant correlation between weight increase from the meal and percentage change in number of drops.

That this response is not modulated into a circadian rhythm was perhaps to be expected, since the weight of the fly does not change across the day, except as a functioh of nutrient loss or gain. The lack of rhythm emphasises that the response is not a reflection of spontaneous locomotor activity, which has been shown (Brady 1972a, 1975) to be modulated by a V shaped diurnal rhythm.

CHAPTER 5

5. MEASUREMENT OF THE OSMOTIC PRESSURE OF HAEMOLYMPH

5.1. Method

Freezing point depression was used as a convenient measure of haemolymph osmotic pressure (O.P.). The apparatus used for the measurement of the freezing point depression was the same as that described by Ramsay and Brown (1955) with cardice as a coolant.

Freezing point determinations were made exactly as described by Ramsay and Brown (1955) for both calibration samples of sodium chloride of known concentration, and haemolymph samples.

Gee (1975 a) had extracted haemolymph by inserting a finely drawn micropipette between the pleural sclerites, but it was found easier here to cut the wing across the remigial cell, when 2-3 drops of haemolymph would form and could be collected as follows. The fly was held immobile by air suction to a pipette applied to the ventral thorax (the pipette being connected to a water pump). A micropipette with one end sealed and treated externally with Repelcote was mounted (open end inwards) with sealing wax into a Pasteur pipette fitted with rubber tubing at its wide end. As soon as possible after cutting of the wing and formation of a haemolymph drop, the sealed end of the micropipette was broken and applied to the drop, so that haemolymph rose by capillary action. The haemolymph was expelled under paraffin in a watch glass whose surface had been made hydrofuge with Repelcote to prevent spreading of the drop. Haemolymph was taken from each fly in this way and stored in a separate watch glass until tested.

Newly emerged teneral males and females were tested to give readings to compare with both newly fed mature flies and mature flies starved for 5 days. A small number of non-tenerals which had been fed only once and then starved for 5 days was also tested.

5.2. Results

Fig.5.1. shows the results of the calibration of the Ramsay apparatus used with NaCl solutions of known molarity. This calibration was used to produce the depression of freezing point estimates given below.

For each group of flies the mean depression of freezing point was calculated. t tests were carried out to establish any significant differences between the groups. This is shown in Table 5.1.

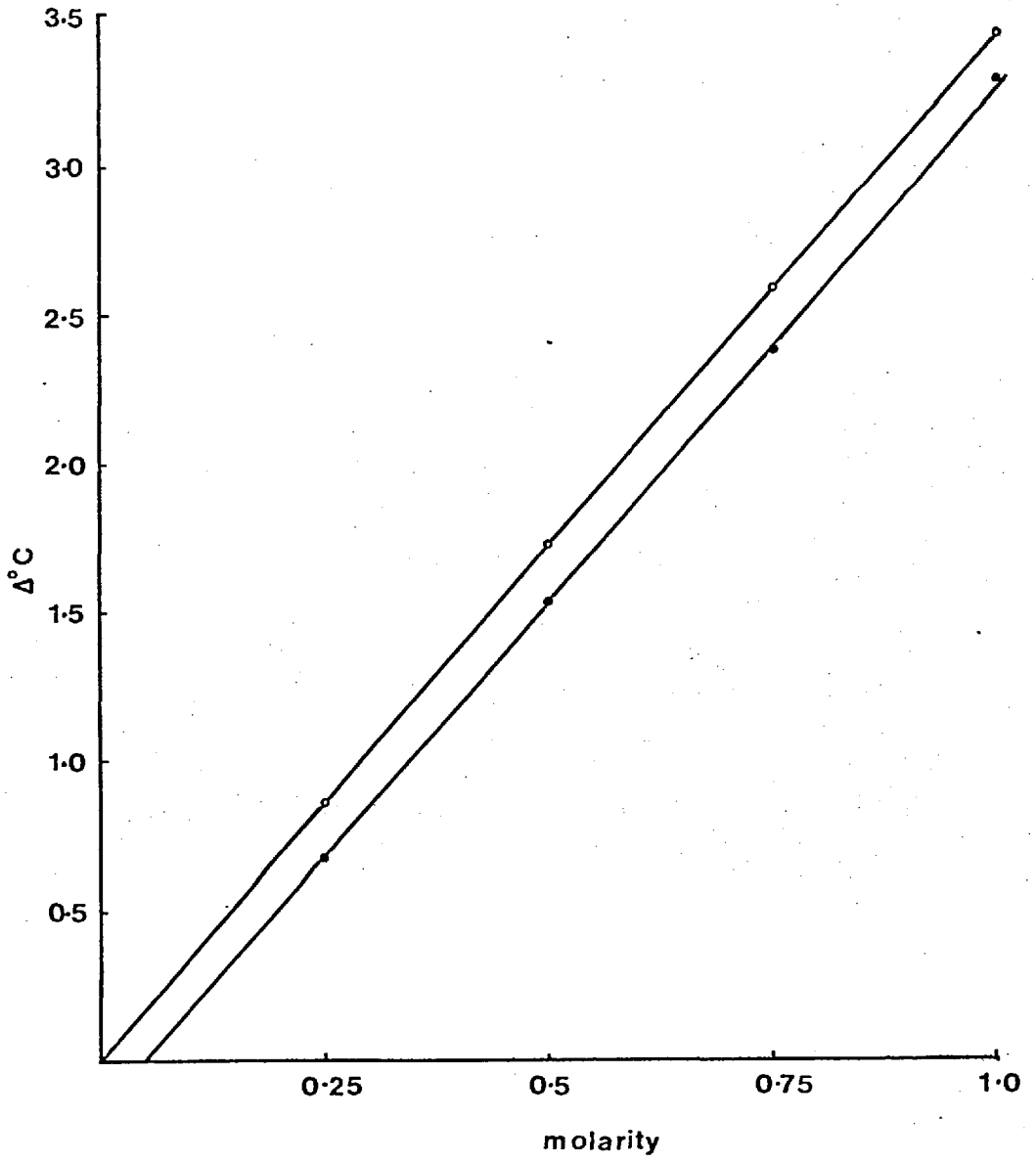
Table 5.1. Mean depression of freezing point for different groups of flies and t tests between the means

Group	N	$\bar{x}\triangle^{\circ}C \pm$ S.E.	t	df	p
Tenerals ♂	4	0.888 ± 0.050	3.13	12	< 0.01
♀	9	0.844 ± 0.039			
Mature flies			3.56	15	< 0.01
♂ day 1	10	1.036 ± 0.023	0.49	18	> 0.1
♂ day 5	10	1.022 ± 0.017			
♀ day 1	8	1.003 ± 0.024	2.27	12	< 0.05
♀ day 5	6	1.367 ± 0.179			

FIG. 5.1. THE RELATIONSHIP BETWEEN DEPRESSION OF FREEZING POINT Δ°C AND MOLARITY OF NaCl SOLUTION

∘, data from the Physical And Chemical Handbook

•, calibration readings



For both males and females, there is a significant increase (see Table 5.1.) in osmotic pressure of the haemolymph from the state in the teneral fly to that of the mature fly. The change appears to be made at the first meal. Four males and two females which had been fed once, and starved for 5 days, gave mean $\triangle^{o}C$ readings of 1.08 and 0.975 respectively, i.e. close to those of mature flies.

For mature males, there is no significant difference between O.P. on day 1 and 5, indicating that the osmotic pressure is regulated in spite of the degree of starvation. For mature females, the difference between O.P. on day 1 and 5 is just significant at the 5% level, but this result should be viewed in the light of the very high standard error of the mean on day 5 compared to that on day 1. Most of this variation came from one fly which had the smallest volume of haemolymph which was slow to exude from the wound.

Throughout all the tests it had been noted that when only a very small volume of blood could be obtained by the standard technique, that sample gave an O.P. reading above the normal. This in spite of the blood being drawn into the capillary immediately any appeared at the wound surface, so that no evaporation would have occurred. These results may indicate that O.P. regulation is not maintained beyond a certain level of starvation and dehydration.

The measurements for tenerals fall within the upper end of the range of commonly found values given by Sutcliffe (1963) of between 0.5 and 0.9 $\triangle^{o}C$, and compare with figures of Gee (1975a) for teneral Glossina austeni of 0.7 - 0.8 $\triangle^{o}C$. The figures for the adults of 1.0 - 1.3 $\triangle^{o}C$, lie at the upper end of the range of values found for other insects.

5.3. Discussion

For the mature flies, it appears that the O.P. of the blood is regulated until changes occur at a late stage in starvation, such changes probably being associated with dessication. This is in agreement with the review of Wyatt (1961) that regulation of haemolymph O.P. is the general rule in insects. Certainly, for the tsetse fly, O.P. of the haemolymph does not change in a parallel way to spontaneous locomotor activity across starvation.

The significant change in haemolymph O.P. from the teneral state to that of mature flies is at first glance contrary to the findings of Gee (1975a) that in spite of fluid equivalent to 80% of the fly's unfed weight passing through the haemolymph of teneral flies during the first hour of diuresis, there is little disturbance to the O.P. of the haemolymph. However, whilst Gee's recordings were of the first hour of diuresis, the present change is recorded between the unfed state, and the state on day 1 after the third blood meal, i.e. more than 24 hours after the meal. Digestion, fat synthesis, and proline level increase consequent to the meal are not near completion until about 12 hours after the meal (Langley, 1966b, and Bursell et al., 1974), and thus the changes between teneral and mature recorded here may be a result of digestion of the first blood meal, not apparent during the initial phase of rapid diuresis.

These findings do not rule out the possibility that the O.P. of the haemolymph is regulated whilst concentrations of some of the constituent compounds or ions alters, and that changes in concentration of these may be a causal factor in change in locomotory activity.

CHAPTER 6

6. ASSESSMENT OF ANY INFLUENCE OF CROP EXPANSION, ABDOMINAL STRETCH OR WEIGHT GAIN ON SPONTANEOUS LOCOMOTOR ACTIVITY

6.1. Tenerals

6.1.1. Method

In an attempt to prevent expansion of the crop with air during wing extension, three different methods were tried. Application of molten beeswax to the end of the haustellum failed to prevent wing extension, so it was necessary to try puncturing the ptilinum. Flies were able to heal the wound made by an entomological pin and continue to extend their wings, although the puncture always caused emission of a drop of haemolymph. Finally it was found that a 1 mm. cut into the ptilinum using a piece of mounted razor blade always caused emission of a drop of haemolymph, and that more than half the flies were unable to heal the wound and complete wing extension, and their abdomens remained shorter than those of flies with extended wings.

To prepare flies for testing, they were picked up within seconds of emerging from the puparium case, and the ptilinum cut. Flies which extended their wings were used as controls. Flies with unextended wings were used as the experimental group which had not undergone crop expansion (Langley 1967b). Both groups had lost a similar amount of haemolymph. It was not possible to record the weights of the newly emerged flies, since the usual anaesthetic used, carbon dioxide, causes crop expansion.

Both groups of flies were tested for spontaneous locomotor activity from lights-on the day after emergence until death. The flies capable of flight were tested in the usual sized actograph, and those capable only of walking/jumping, in the small actographs, so that the shorter distances walked would rock the box.

6.1.2. Results

Only data from flies which lived at least two days after the day of emergence were used to calculate the mean daily activity as a percentage of the total. The means for the two groups are given in Table 6.1. and Fig. 6.1.

Table 6.1. Mean daily activity expressed as a percentage of the total ± S.E.

DAY	Flies with unextended wings	Flies with extended wings
1	48.53 ± 5.09	27.06 ± 5.35
2	38.84 ± 3.66	27.22 ± 3.82
3	6.47 ± 2.31	26.94 ± 3.88
4	3.53 ± 2.04	14.94 ± 3.31
5		3.78 ± 1.65

For 19 tenerals with unextended wings, hereafter called W1, and 18 with extended wings, hereafter called W2, a t test was performed on the total numbers of flight bursts shown from insertion into the actographs until death. This gave $t = 0.25$ at 35 df with $p>0.8$. Since there is no difference in the overall number of bursts, the percentages of activity per day are comparable.

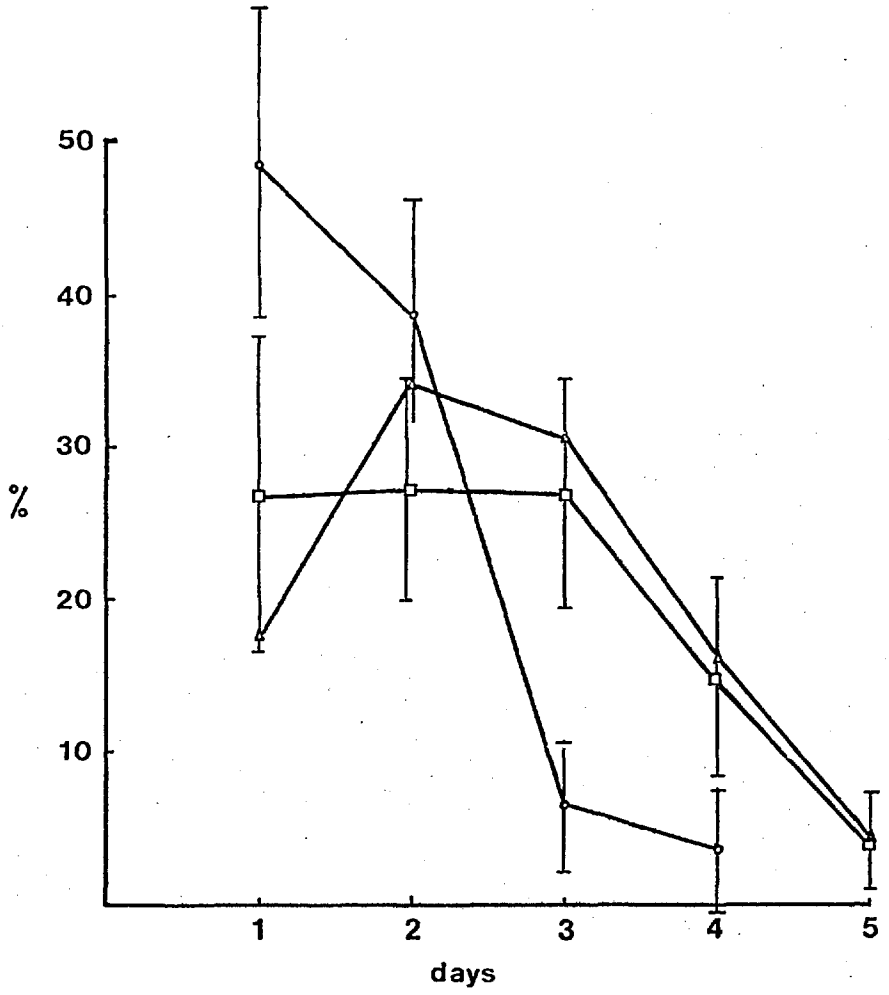
There is a clear difference between the groups, W1 flies being most active on day 1, with a progressive decline in activity on subsequent days, whilst W2 flies show a lower activity level on day 1, which is maintained for a further two days before activity begins to decline.

FIG. 6.1. THE EFFECT OF PTILINUM PUNCTURE AT EMERGENCE ON SUBSEQUENT ACTIVITY OF TENERAL FLIES.

°, W1 flies, unextended wings N=19 □, W2 flies, extended wings N=18

Δ, untreated flies N=6

Bars=95% confidence limits. Ordinate: flight bursts per day as a percentage of the total number across starvation



The walking/jumping behaviour of the W1 flies would have involved an unknown, but lower energy expenditure than the flight of W2 flies. During flight flies have a metabolic rate 100 times that of flies at rest, (Bursell et al; 1974). A comparison of the length of bursts shown on day 1 by 8 flies chosen randomly from each group gave a difference between the two means (which were 0.685 mm. length pen marks and 0.618 mm. respectively) as less than 0.0869 standard deviations from zero, with an associated probability of $p > 0.92$. Thus in spite of the different modes of locomotion, and different levels of energy expenditure, spontaneous bursts of locomotor activity of closely similar lengths were performed by each group of flies.

6.1.3. Discussion

Since the number and length of bursts of locomotor activity performed, and the amount of haemolymph lost at ptilinum puncture, are similar for the two groups of fly, the difference in the change in activity across starvation for the two groups may be explained as follows. During wing extension in normal flies the crop expands with air (Langley 1967 b). This expansion may be measured by crop or abdominal stretch receptors and relayed to the CNS to set the high initial threshold for spontaneous activity. Subsequent threshold lowering is probably related to change in weight (see Brady, 1975, for mature males).

The threshold of W2 flies is not set so high as for untreated flies due to difficulty in fully expanding the crop immediately after ptilinum puncturing. However, it is considerably higher than that of W1 flies and the course of subsequent activity, especially the post peak decline is more similar to that of untreated than to that of W1 flies. W1 flies, which had no crop expansion did not have the threshold raised, and performed so many bursts on day 1 (with a mean of 49.74 ± 8.84 bursts per fly, compared to 21.72 ± 4.84 of W2 flies) that nutrient shortage causes a reduction in activity level the next and following days in spite of the (assumed) lowering of threshold due to weight loss.

Thus it appears that in teneral flies crop expansion sets the threshold to a high level with the result that as the threshold lowers, probably in association with weight loss, there is enough nutrient left to support a rise in activity for 2-4 days. Such an increase before nutrient shortage reduced activity was observed in untreated flies shown here, and by Brady (1972a).

6.2. Mature flies

6.2.1. Method

Dilute blood meals were given through a membrane feeding system.

Blood was removed from the mid vein of an ear of a lop-eared rabbit by a technical assistant. 7.5 ml. of blood were drawn slowly into a 10 ml. disposable syringe and added to 1.5 ml. sterile sodium citrate solution (made by adding 6 gm. to 100 ml. distilled water) to prevent coagulation. The citrate diluted whole blood to 83% of its original strength.

All further materials and equipment used were sterilised in an autoclave unless otherwise stated. 6 ml. of the blood were poured into one small glass tube, and this is referred to as 83% blood hereafter. 3 ml. of the blood were poured into another tube, and to this were added 3 ml. of 0.85 M NaCl to give a 50% dilution of the first sample which reduced its concentration to 42% of whole blood. The addition of 0.85 M NaCl retained the same osmotic pressure as whole blood (Langley, 1966 a). Yorke and Blacklock (1915) had found that Glossina palpalis would readily imbibe blood diluted to 50% strength by addition of normal saline.

The blood was maintained at 37°C during feeding as follows. Petri dish lid containers were placed on the bottom of a pyrex glass domestic casserole dish floating on a water bath at 39°C.

A thin layer of absorbent cotton wool was placed in each petri dish, and the different blood dilutions poured singly into each dish.

Thin metal rings 8.7 cm. in diameter, and 13 mm. deep had been cut from a cylinder. The sharp edges had been rounded by plastic sleeving wire. A square of two-way stretch Parafilm M was sterilised by wiping with 70% alcohol, and was gently heated and stretched and pulled over the metal ring until firmly in place. The ring just fitted inside the Petri dish lid, and was placed on top of the blood and cotton wool, Parafilm side down.

1 mm. thick Agar membranes, made in the base of a Petri dish with 3% Agar dissolved in 0.15 M NaCl (Langley, 1972), were placed on the parafilm inside the metal rings. Within minutes of being positioned, the membranes were warm enough to elicit probing.

Flies used for these experiments were given their first two blood meals from rabbits' ears as normal. The membrane feeds were given on the same day that the normal third meal would have been given. During the feed the plastic lid of the tube was replaced by nylon netting held in place by a rubber band. This prevented an accumulation of moisture at the top of the tube. Care was taken to return the correct lid to each tube for weighing purposes.

The weight of each fly before the meal was measured by weighing a standard tube empty, and then with the fly inside. The weight of the meal was measured immediately after imbibition, and at various intervals afterwards, the time at which each weight was taken being noted.

At each run of this experiment, 3 flies that had fed on 42% blood and 3 that had fed on 83% blood were placed singly in rocking

box actographs the evening after the membrane fed meal, and their activity recorded until death. Flies with nutritional backgrounds as closely similar as possible were used. Any remaining flies were monitored for weight loss until death.

6.2.2. Results

The weight loss during rapid diuresis was monitored to show any difference in rate between the two blood dilutions, and to show the final percentage of the blood meal retained in each case.

Fig.6.2. shows the percentage weight retained by the two groups from the time of feeding until 240 minutes after the meal. Since weight measurements of each fly were made at different times, it was necessary to estimate each fly's weight at the specific times selected for calculating the means. This was achieved by calculating for each fly, the weight loss per minute between successive measurements and estimating the loss of weight from this to match the specific time required. The means of these estimated weights are expressed as a percentage of the blood meal retained, and are plotted on Fig. 6.2. From this it can be seen that the rate of diuresis is the same for each group up to 30 minutes after the feed. After 30 minutes, the rate of diuresis in flies fed 83% blood slowed down more quickly than those fed on 42%. Both groups had ended rapid diuresis within two hours. By the end of diuresis, 42% flies had retained 29% of the original blood meal intake, and 83% flies had retained 49%. There was no significant difference between the mg. imbibed/mg emergence weight between the two groups (see below), so the retention figures are comparable.

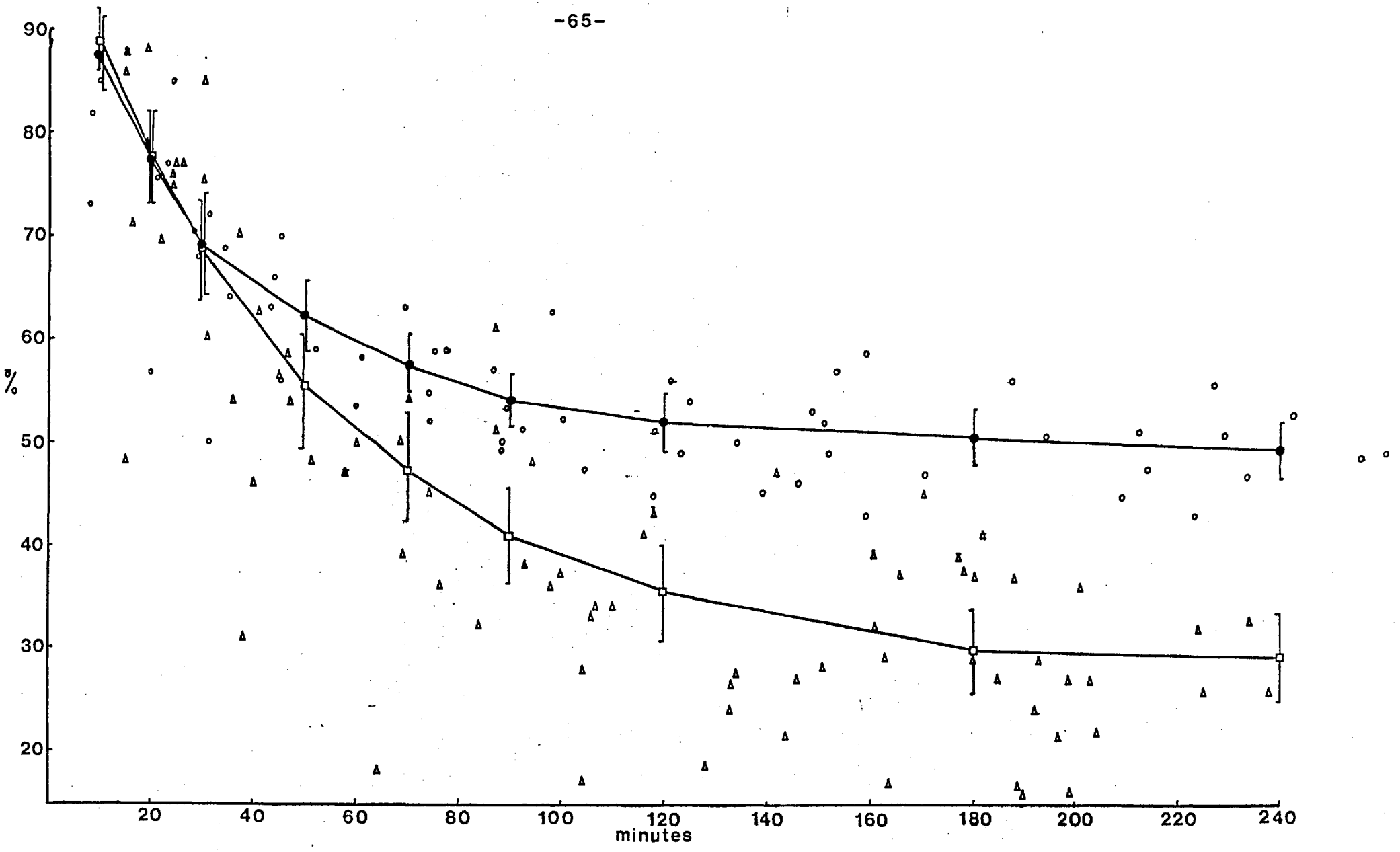
All results given below are corrected to mg. imbibed per mg. emergence weight to standardise the results. A small proportion of the flies, particularly those fed on 83% blood, failed to complete

FIG. 6.2. THE PERCENTAGE OF THE BLOODMEAL REMAINING DURING RAPID DIURESIS IN FLIES FED DIFFERENT BLOOD DILUTIONS.

•, ●, Individual readings and means respectively of flies fed 83 % blood. N=14

▲, □, Individual readings and means respectively of flies fed 42 % blood. N=21

Bars = 95 % confidence limits


90
80
70
60
%
50
40
30
20
20
40
60
80
100
120
140
160
180
200
220
240
minutes

diuresis successfully and were discarded. Data from flies in the actographs were used to calculate total daily activity when 42% flies had lived for at least 3 days, and 83% flies had lived for at least 4 days, after the meal.

The flies chosen to be placed in the actographs were tested to confirm that there was no difference between the two groups in size of blood meal taken. This gave $\underline{t} = 1.03$ at 31 df with $p>0.1$, and thus no significant difference.

The data from the flies in the actographs were tested for any difference in the total number of flight bursts per fly across starvation in the two groups. 83% flies gave a mean of 31.16 ± 3.85 flight bursts, and 42% flies gave a mean of 21.16 ± 2.81 bursts. The $\underline{t}$ test between the two groups gave $\underline{t} = 2.15$ at 30 df with $p<0.05$, so a significant difference in the number of bursts was shown between the groups.

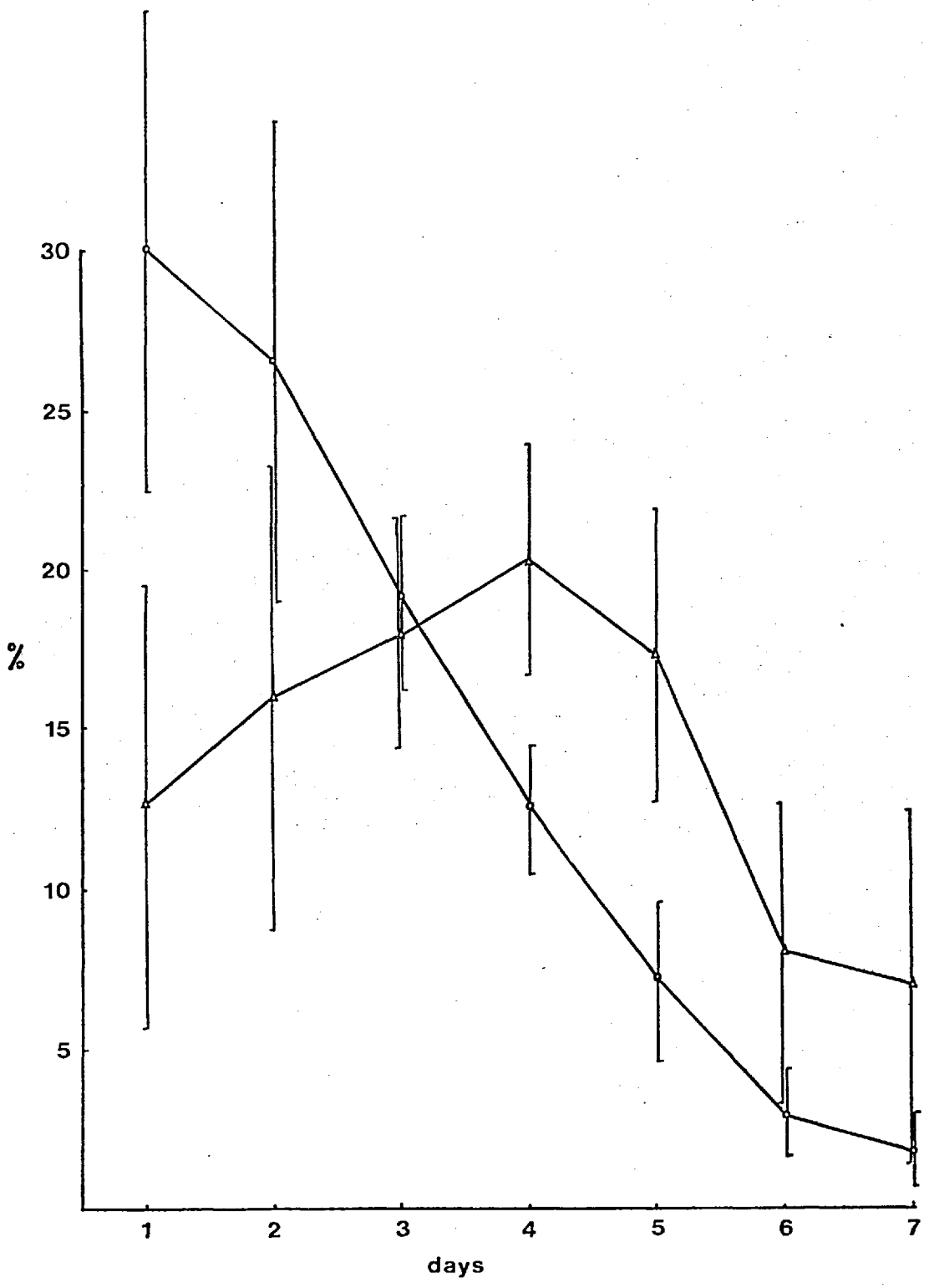
Fig. 6.3. shows the means of the number of bursts per day expressed as a percentage of the total number of bursts across starvation for each group. There is a clear difference between groups, with 42% flies most active at the beginning of starvation and then activity progressively declining through to death. 83% flies showed a steady increase in activity up to day 4, and a progressive decline thereafter.

A $\underline{t}$ test was performed on data of third blood meal sizes from all flies fed, including those used for actographs, to show any compensation by the flies for the different levels of dilution. 28 42% flies gave a mean of 2.24 ± 0.11 mg./mg. emergence weight and 37 83% flies gave a mean of 2.25 ± 0.11 mg./mg. emergence weight, the two groups giving a $\underline{t} = 0.09$ at 63 df and $p>0.9$. There was therefore no significant difference in the size of the blood meal taken by the two groups.

FIG. 6.3. THE EFFECT OF DIFFERENT BLOODMEAL DILUTIONS ON SUBSEQUENT ACTIVITY OF FLIES

Δ, FLIES FED 83% BLOOD. N=14. ○, FLIES FED 42% BLOOD. N=18.

ORDINATE: FLIGHT BURSTS PER DAY AS A PERCENTAGE OF THE TOTAL NUMBER OF BURSTS. BARS=95% CONFIDENCE LIMITS.



6.2.3. Discussion

Whilst the blood imbibed by the 83% flies contained twice the amount of nutrient of that taken by 42% flies, the 49% (see Fig. 6.2.) that they retained is not double the 29% retained by the 42% flies. This was unexpected since Bursell (1960c) indicates that flies retain enough moisture at each meal to make up the tissue deficit. Thus, given comparable deficits, the 83% flies would have had to retain a greater proportion of the meal to ensure hydration comparable to flies fed on 42% blood. No explanation for this is immediately apparent.

Within 30 minutes of commencing diuresis, both groups had lost 33% of the initial meal intake, this is only slightly less than the 38% loss in 30 minutes reported by Moloo and Kutuza (1970) for G.brevipalpis. These workers found that flies fed on different meal sizes showed the same rate of diuresis, which is thus independent of meal size. From the results shown here for G.morsitans, since flies fed on different dilutions of blood show the same rate of diuresis, then this rate is also independent of dilution of the blood meal. The rate of diuresis must reflect the rate of crop emptying, so that this is also independent of dilution, in contrast to the situation in Phormia regina, where the rate of emptying is faster the more dilute the contents (Gelperin, 1966 b).

Rice, Galun, and Margalit (1973 b), have commented that the preponderence of mechanoreceptors and the limited range of effective phagostimulants of G.austeni reflect the obligatory nature of the blood sucking habit in the fly. That there is no mechanism whereby the crop responds to different dilutions of the blood is a further reflection of the single food source of the tsetse.

Within three hours the 83% flies had lost 51% of the initial meal weight, which is very similar to the 53% voided in three hours by mature male G. morsitans reported by Brady (1975).

83% flies performed only 1½ times as many flight bursts as 42% flies, in spite of receiving twice the amount of nutrient. Since control flies showed no change in flight burst length across starvation (see also Brady, 1975), the smaller number of flight bursts by 83% flies in relation to their solids intake could have resulted from extra energy utilisation per flight due to extra weight.

The difference between the two groups in change in activity across starvation indicates that it is not the initial stretch of the crop and abdomen, which was the same for each group, but that it is the weight retained from, or the abdominal stretch after diuresis and subsequent to the meal, which cause the observed change in threshold to spontaneous locomotor activity. This confirms the finding of Brady (1975) that weight, and abdominal stretch as a function of it, correlated most strongly with change in activity.

These results also compare with those of Hopkins (1964), who showed that in Stomoxys calcitrans the probing response to vapour of flies fed dilute sucrose was very similar to that of an unfed control group. These flies had imbibed larger quantities of the more dilute meal than a similar group fed on concentrated sucrose whose thresholds rose considerably after the meal, and thus she concluded that the probing threshold depends upon the nutritive value not the volume of the meal.

Galun (1975b) had fed tsetses on saline + A.T.P. solutions made more viscous with dextran. This increased the length of time of each feed, but did not reduce the volume taken, and thus the length of feeding is also unimportant in altering the threshold to imbibe.

Mews et al. (1976) found that flies could compensate for blood diluted with 0.85% NaCl up to 40%, and still retain a rate of reproduction comparable with control groups. They found that frequency of feeding and meal size were proportional to the degree of dilution over a period of time. Since there was no significant difference between weights imbibed by 42% and 83% flies, compensation such as that observed by Mews et al. must occur at subsequent meals, and the flies do not respond to dilution per se. but to the resultant lack of nutrition.

In addition to this, Rice (1972b) found that tsetse flies with an incision in the ventro medial abdomen and crop to allow the blood to flow out, and thus with no abdominal stretch from the intake of blood, imbibed twice the usual weight. He concluded that the satiety response depends primarily on stretch reception and only secondarily on adaptation or fatigue of cibarial sense organs. That both groups here imbibed the same amount of blood confirms this, since if adaptation was primarily important in cessation of imbibition then it would be expected that smaller meals of the more concentrated blood would be taken. This is not the case.

CHAPTER 7

7. ASSESSMENT OF ANY CENTRAL EFFECTS FROM THE ACT OF FEEDING, BY PARTIAL FEEDS

7.1. Method

Flies with known equivalent intakes at their first two blood meals were split into two groups. The first group, acting as control, was not offered a third meal, while the other group was offered a rabbits' ear to feed on in the usual way. This latter group was allowed to respond to the temperature/humidity gradient and alight on the ear, go through the exploratory tasting part of probing, and begin imbibition. They were then removed from the ear as soon as their abdomens showed the first tinge of red. The intake of blood was measured, and partially-fed and non-fed flies were placed in actographs and their spontaneous locomotor activity measured from lights-on the next day until death.

The first groups of flies were offered a partial feed at the same time as they would normally have been offered the third blood meal, i.e. after three clear days had elapsed since the second blood meal. This resulted in recording beginning on the morning of the fifth day since the second blood meal.

Results from the early groups showed parallel changes in decrease of activity between the partially and non-fed groups. But in view of the length of time since the previous full feed, this did not rule out the possibility that the similarity was due to shared shortage of nutrient rather than lack of effects from the act of feeding in the partially-fed group. Shortage of nutrient

is likely to be important in flies of this age, since, as well as various metabolic demands on energy from meal intake, 15% of the energy from the first two meals is used in protein development of the thoracic musculature and cuticle (Bursell et al., 1974)

To investigate this further in a second group, flies were given a partial meal (or not fed) on day 2, i.e. when only 1 clear day had passed since the second blood meal. These were recorded for spontaneous locomotor activity as before.

7.2. Results

When testing for any effects from the act of feeding, it was thought inadequate to know merely the change in percentage activity each day, since two similar levels of percentage activity might conceal different absolute activity levels. Central effects from the act of feeding, if present, may reasonably be expected to cause a reduction in activity level which in itself must influence any subsequent change in activity level, and thus, the percentage of activity performed each day.

In view of this consideration, the results are presented as the mean number of flight bursts per day. Whilst there is considerable variation in the amount of activity performed by different flies (Brady, 1972a), groups of flies chosen in the same way from the population of flies sent weekly from Bristol should all contain a similar range of activity levels. Thus it is considered that a meaningful comparison may be made between the mean activity levels of different groups.

In order that any observed differences in activity level could be attributed to the effects of the experimental feeding treatments,

the mean weight of blood imbibed during the first two preparatory blood meals of the different groups of fly was calculated. This was expressed as mg blood imbibed/mg emergence weight for the total of the two meals, for each experimental group. t tests between each pair of groups showed no significant difference, with $p > 0.1$ in all cases.

The means ($\pm$S.E.) of the number of flight bursts performed each day by each group after the experimental partial or full third blood meal, are given in Table 7.1., and shown in Fig.7.1.. Days here refer to the days following the experimental third 'meal'.

Hereafter the following letters are used to denote the experimental groups:- partially fed (PF), non fed (NF), and fully fed (FF). The number following indicates the day after the second blood meal on which the experimental meal was given.

PF4 flies, with a mean experimental blood meal intake of 4.25 mg, and 0.28 mg/mg emergence weight, gave 27% fewer flight bursts on day 1 than NF4 flies. FF4 flies, with a mean intake of 41.79mg, and 2.06 mg/mg emergence weight, performed 33% fewer flight bursts on day 1 than the NF4 flies. PF2 flies, after a mean intake of 7.36 mg, and 0.36 mg/mg emergence weight, performed 32% fewer flight bursts on day 1 than NF2 flies. It should be noted that although PF2 flies imbibed nearly twice the blood of PF4 flies, this probably offered similar or a smaller percentage change in their weight, since PF2 flies would have had a greater proportion of the second blood meal remaining. Thus, there is not a simple relationship between mg blood imbibed, and resultant change in activity level. All three fed groups exhibited similar levels of activity on day 1, irrespective of the volume of blood imbibed.

Table 7.1. MEAN NUMBER OF FLIGHT BURSTS PER DAY ± S.E.

	Treated day 2		Treated day 4		
Day	Partially-fed N=15	Non-fed N=18	Partially-fed N=21	Non-fed N=19	Fully fed [N=77 days 1-3]
1	11.60±0.94	17.17±2.44	11.19±1.31	15.26±1.79	10.08±0.67
2	13.33±1.19	18.22±2.40	11.29±1.23	13.68±2.65	12.29±0.80
3	15.53±1.23	17.44±2.00	11.38±1.30	7.15±1.73	15.35±0.85
4	15.73±1.27	16.00±1.76	7.00±1.32	2.69±0.99	17.87±1.30)
5	13.50±1.88	7.89±1.45	1.52±0.53		22.73±2.04) N=15
6	6.07±1.57	2.78±0.88			
7	1.53±0.9				

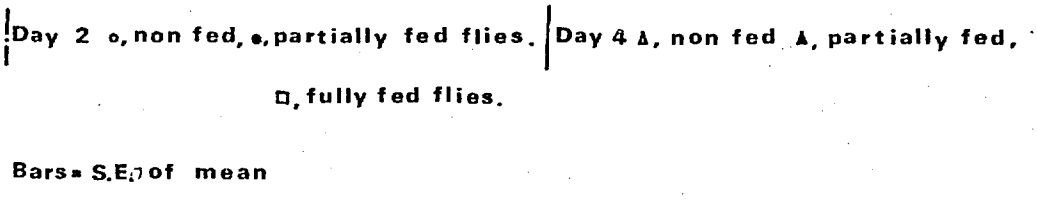
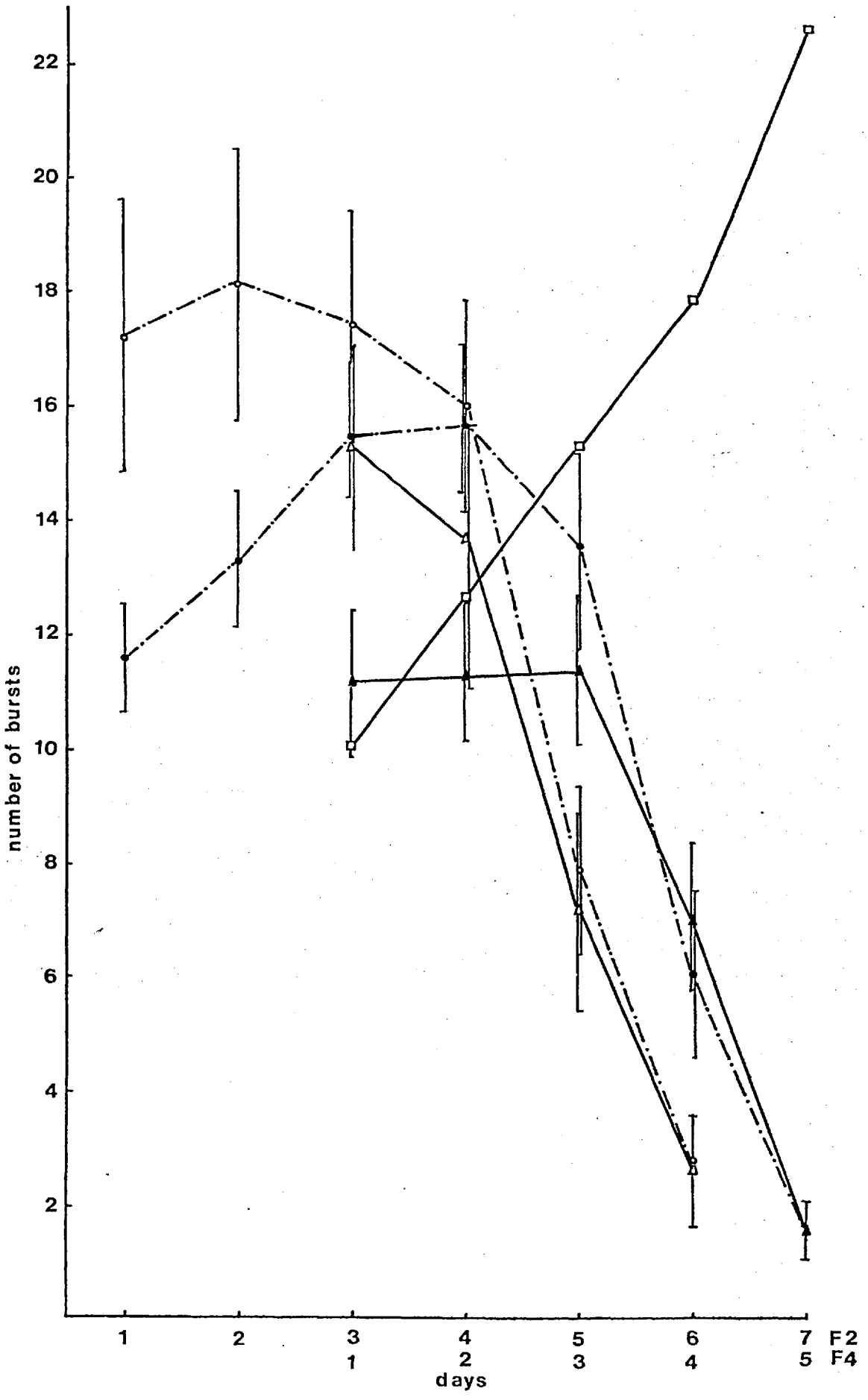


FIG. 7.1. MEAN NUMBER OF FLIGHT BURSTS PER FLY EACH DAY SUBSEQUENT TO DIFFERENT SIZES OF BLOODMEAL


22
20
18
16
14
12
10
8
6
4
2
number of bursts
1
2
3
4
5
6
7
F2
1
2
3
4
5
F4
days

The mean number of flight bursts of 77 FF4 flies on day 1 of 10.08 ± 0.67, is comparable to 8.9 ± 0.9 bursts for 22 mature males reported by Brady (1972a), although the activity of his flies was recorded in actographs twice the volume of those used in the present experiment. His recording also began some 6 hours nearer to the meal than did the present recording. There is close similarity between the number for FF4 flies, and 11.6 ± 0.94 for PF2 flies and 11.19 ± 1.31 for PF4 flies.

Whilst PF4 flies showed 27% less activity on day 1, than NF4 flies, this did not leave sufficient nutrient to enable flies to give a substantial increase in activity on the following days. PF2 flies showed less activity on day 1 than NF2 flies, and a peak on days 3-4. This was 1-2 days later than the peak of the NF2 flies. The same is true of the relationship between PF4 and NF4 flies, and the ability to increase the level of activity probably reflects both the small amount of extra nutrient available from the partial feeds, and that nutrient saved by the drop in activity immediately after the meal.

The activity of the different groups of flies began to decline from different peak activity levels. However, the rates of decline are so similar that it appears that, for each peak activity level, the decline begins at a level of nutrient sufficient to support a standard rate of decline. It can be assumed that the activity of FF4 flies would have begun to decline after day 5 (Brady, 1972a), with the decline resembling the closely similar declines of all 4 groups of experimentally 'fed' flies.

It would be expected that NF4 day 1 and NF2 day 3 would have shown similar levels of activity. From Fig.7.1. it appears that NF2 day 3 flies are more active. However, there are relatively large standard errors to the means, and a *t* test gave no

significant difference, with $\underline{t}$ = 1.21 x 35 df with $p > 0.2$.

The standard errors of the means of non-fed groups which were placed in the actographs at different time lags after their second meal are rather large. This may reflect the variation within a group of flies in the number of flight bursts performed, and thus the varying rates of nutrient utilisation and weight loss for different flies. By contrast, the standard errors of the partially 'fed' flies, all placed in actographs immediately after the experimental 'meal', were much smaller. As with the similar activity levels, the lower S.E.'s on day 1, must indicate a standard imput from the act of feeding, irrespective of the weight imbibed.

To check that the number of bursts was not maintained at the expense of a reduction in burst length at later stages in starvation, the following test was performed. The Edgecumbe Peebles output was carefully analysed to look for any change in the length of flight bursts after each fly's peak in activity level. The length of each burst has been shown to remain nearly constant up to the peak, both in the apparatus under discussion (see Table 3.1), and by Brady (1975,p.809). Ten flies were used which showed at least one day of lower activity before the peak was reached. The day of the peak was called day 1, and lengths of bursts were measured on it and on days following until death occurred.

Lengths of flight bursts were noted by measuring the width of pen markings on the pen recorder output. Only flight bursts from dawn to the end of the morning activity were measured, but there is no change in flight burst length across the day (Brady and Crump, 1978).

The mean length of each fly's bursts on each day was calculated, and a $\underline{t}$ test performed between lengths on days 1 and 2, 1 and 3, and 1 and all lengths shown on the day before death. These results are shown in Table 7.2.

Table 7.2. MEAN LENGTHS (mm OF PEN MARK) OF FLIGHT BURSTS

Fly	Day 1	2	3	4	5
1	0.539	0.857	0.433		
2	0.517	0.565	0.613		
3.	0.891	0.607	0.433		
4	0.564	0.520	0.400		
5	1.164	1.060	0.820	1.020	0.575
6	0.900	0.300	0.200		
7	0.647	0.578	0.489	0.300	
8	0.755	0.847	0.840	0.520	
9	1.230	0.730	0.533		
10	0.982	1.040	0.718	0.673	0.433
GROUP MEAN ± S.E.	0.819 ±0.08	0.710 ±0.08	0.548 ±0.06	Final days before death 0.444 ±0.04	

	Days 1 + 2	Days 1 + 3	Day 1 and final day
t tests	$t = 0.97$ df = 18 $p > 0.3$	$t = 2.62$ df = 18 $p < 0.02$	$t = 4.14$ df = 18 $p < 0.01$

Although there is a small reduction in flight burst length from the day of peak activity to the following day, this is not significant, with $p > 0.3$. Significant difference is not reached until the third day after the peak, which for 60% of the flies was the day before death. The significance is increased by comparing all day before death lengths with the lengths on the day of peak activity. Thus it appears that flight bursts of standard length continue to be performed until shortly before death.

7.3. Discussion

In spite of the different sizes of meal imbibed by PF2, PF4 and FF4 flies, all three groups showed a very similar level of activity on day 1 after the meal, but followed different trends after this. Thus there appears to be a base line level of activity reached after meals of any size, with the length of any subsequent increase in activity and the level reached reflecting the levels of nutrient remaining.

This apparent setting of a base line level of activity following a meal, irrespective of the size of that meal was unexpected, in view of the strong negative correlation between weight and activity levels found by Brady (1975). From that weight-activity correlation, it was to be expected that FF4 flies with a blood meal intake, as mg/mg emergence weight, 7.4 and 5.7 times the intake of PF4 and PF2 flies respectively, would have given activity levels appropriate to the different weights of flies after the meal. This is not the case.

For flies with adequate nutrient, such as those in PF2, activity reached a peak level, followed by a progressive decline until death. However, weight would have continued to decrease from the time of feeding to that of death. It is difficult to envisage a threshold of activity being set by a bimodal response to weight, so that beyond a certain weight, further decrease inhibited activity. Furthermore, another measure of responsiveness, probing tendency, has been shown to increase until hours before death. (See Chapter 8.).

Bursell *et al.* (1974), comment that the progressive increase in the proportion of time spent active during a hunger cycle shown by mature males, as reported by Brady (1972a) correlates well with the progressive increase in the proline reserve of mature flies, during the first three days after the meal, although there are no

published data showing this. They give the observation of Hargrove (1973) that the progressive decline in proline concentration during flight is associated with a corresponding fall in wing beat frequency as an indication of 'the close relation between proline availability and flight activity' within a flight burst. Since it has been implied that proline may control flight activity, any feasible method by which the association may be maintained should be examined.

There is a considerable metabolic cost in digesting the blood meal. Bursell (1970) described the following costs. Energy is lost in the synthesis of triglycerides and to a lesser extent (except for during the initial growth of flight muscle) proteins from the amino acids released, and in the detoxication of α amino nitrogen by incorporation into uric acid. Energy is also used for transport of digestive products and recycling water and inorganic salts through the malpighian tubules and the rectal gland system. Because proline is the main substrate for oxidative metabolism, both the 6 hours of intense oxidative (digestive and excretory) activity after the meal, and the following lipogenic period are characterised by a very low proline level. The important result of this activity is that during day 1 after the blood meal, the proline concentration from thoraces of resting flies falls to half the pre-meal level, and does not regain the pre-meal level until some 30 hours later. In the experiment under discussion, recording of activity began the day after the third meal, some 20 hours after the meal.

The amount of trypsin found in the digestive part of the midgut correlates with the protein level there (Gooding, 1974b). In addition, digestion ceases at about 30 hours. (Langley 1966b, McCabe 1973), so that there will be a constant length of time taken to digest any size of meal, and during the first day proline synthesis should be matched by proline utilisation (for energy consuming processes described) for all sizes of meal.

The change in proline levels and synthesis across starvation are described by McCabe (1973) as follows. Abdominal proline increases slightly up to 48 hours after the meal, and thoracic proline (which forms 85% of total fly proline) reaches the highest concentration at 72 hours after the meal and showed only a very slight decline to 96 hours.

The methods of proline synthesis change across starvation. There are two de novo methods of synthesis. In the earliest, amino acids released from the blood meal early during digestion may be incorporated directly into proline. This method of production ceases when digestion ceases at 30 hours. Proline may also be synthesised from abdominal lipid sources, with the residual blood meal (RBM) used to supply free C. This is a rapid method of synthesis, and is thought to maintain proline supplies during flight (But see Bursell, 1978). In addition, proline is resynthesised from alanine produced as a result of partial oxidation of proline. This is a much slower process, which on days 2, 3 and 4 requires 45 minutes for proline to approach the pre-flight levels after 4 minutes of induced flight (Bursell, 1963). After exhaustion of the RBM, each flight adds to a nitrogen shortage, and proline is then resynthesised by a reversal of the oxaloacetate decarboxylase enzyme (Unpublished data by Bursell and Hargrove, quoted in McCabe 1973).

If proline concentration was setting the activity threshold, it would be expected that the decline of proline after days 3-4, and the change in methods of resynthesis of proline after the exhaustion of the RBM on day 4 would be reflected in a change in activity level. However, caution is needed in comparing observed activity with proline levels, since although data in McCabe (1973) were collected from flies at similar temperatures to those used to record activity, his flies were kept in constant dark, and so would have been very inactive (Brady, 1972a). Had McCabe's flies been in a L.D. 12:12 hour cycle, and in actographs,

it can be assumed that due to an increase in activity the observed decline in proline level, after the maximum on days 3-4, would have occurred earlier, reflecting the energy utilisation due to the increased activity. The data for exhaustion of RBM came from Brady (1975), from flies under similar conditions and which had imbibed similar amounts of blood per mg emergence weight to those used for activity measurements here. There is a smooth increase in activity in FF4 flies during days 3, 4, and 5, in spite of the decrease in proline level described above, indicating there is an an overriding factor other than proline influencing activity.

Furthermore, it is difficult to envisage a behavioural threshold being set by a metabolite whose concentration fluctuates with each flight burst, dropping to 17% of the original level in 2 minutes of flight, and requiring approximately 45 minutes to approach the pre-flight level after the flight (Bursell, 1963).

The following hypothesis is offered as a means of reconciling the differences in timing between the change in proline and change in activity across starvation, the strong correlation between weight and activity (Brady, 1975), the similar levels of activity of flies of different weight on day 1 (Fig.7.1.), the maintainance of a standard burst length until late in starvation and the decline in number of bursts late in starvation.

The day after the experimental 'meals', due to the metabolic activity described, the resting proline levels of 'fed' flies will all have been very low irrespective of different weights imbibed. It is envisaged that this low level would have been close to the minimum level needed to sustain a flight burst of standard length. This would mean that a new flight burst would not be initiated until the proline reserve was reconstituted to its maximum resting level for day 1. This would reduce the number of flight bursts in all 'fed' groups, with proline as a limiting factor explaining the similarity between the groups.

After day 1, which set activity to a base-line level, activity in FF4 flies did not reach a peak at the same time as proline would have reached its highest concentration. This must indicate that proline, although it may act as a limiting factor, is not excitatory. The steady post day 1 increase in activity of FF4 and PF2 flies may be explained by the weight-activity correlation, with threshold to spontaneous locomotor activity being set by the fly's weight change.

The decrease in activity in PF2 and PF4 flies after the peak in activity, and the earlier decline in activity of NF2 and NF4 flies may be explained as follows. In spite of continued weight loss, and lowering of threshold, shortage of proline again becomes a limiting factor, reducing the number of flight bursts.

Whilst this hypothesis appears to fit the available experimental evidence, in order to increase confidence in it, the standard errors on the activity data should be reduced by increasing the number of flies in each experimental group.

The observed similarity on day 1 after different sizes of meal might also be due to central effects of the act of feeding itself rather than nutritional changes resultant to the act of feeding. However, this is unlikely since no evidence has been found to indicate this for other insects (Barton Brown, 1975). Hopkins (1964), in a study of Stomoxys calcitrans, showed that the probing threshold was not significantly altered by a partial blood meal. Hudson (1957) showed that the time course of taste threshold change in Phormia regina could be altered by increasing the activity of flies, so that the resultant change in nutrient was of greater importance than any central feeding effects.

CHAPTER 8

8. INVESTIGATION INTO CHANGE WITH STARVATION OF ACCEPTANCE THRESHOLD TO STIMULI FOR PROBING

8.1. Materials

Some high density, load-bearing polystyrene was taken, and a ball of 12.5 mm diameter carved out. The ball was halved and the core scooped out leaving two shells.

A ball of low enough weight, and which could be heated to known exact temperatures was made as follows. An ITT light bulb type 30379 γ was lain across the centre of one half shell, its wires held in position in a very shallow groove. The bulb was covered with silver foil to prevent any light escaping. The other half shell was glued back in position. Just enough wire emerged from the sides of the ball that very fine gold wire could be attached by cold silver solder. These wires were attached to two terminals 4" apart, supplied from a variable voltage output. The ball weighed 290 mg.

The ball was calibrated as follows. A DANA digital voltmeter model 4430 was prepared for calibration. An ITT unmounted bead type thermistor U23US of 250μ diameter was connected to a battery of known voltage, and then into the voltmeter via resistor of 500R type W21.

The thermistor was then calibrated at ambient temperatures of 25 $\pm$ 1°C, by being lain against the bottom of a glass dish floating on a water bath of known temperature. The temperature

of the water bath was raised very slowly, and the volt reading noted at each temperature. Each temperature was tested on 2 separate runs, and a mean noted. Table 8.1. gives the relationship between voltage recorded, and temperature of the water bath.

The thermistor was then used to monitor the temperature change at the surface of the ball, when different voltages were passed through the inner light bulb. Voltages from 1 V - 9 V in 1 V increments were tested. The thermistor was lain against the side of the ball in the same way and at the same ambient temperature as it had been calibrated against the glass dish. With the voltage being raised 1 V at a time, the temperature at the outside of the ball had stabilised within $3\frac{1}{2}$ minutes of the voltage change. A series of voltmeter readings from 4 minutes to 8 minutes were taken. Table 8.2. gives the relationship between the voltage input to the ball, and the voltmeter readings from the thermistor. For each voltage input, at least 19 voltmeter readings were recorded. The means of these recordings are given in the table.

Fig.8.1. shows graphically the data in Tables 8.1. and 8.2., and was used to find the voltage input to the ball needed to give the desired temperature at the surface. Table 8.3. shows the relationship found, and those voltages used in experiments.

Table 8.1. Relationship between voltmeter readings (means of 2 measurements) and temperature

°C	25	26	27	28	29	30
Voltmeter reading	0.11014	0.10767	0.10590	0.10256	0.09915	0.09697
°C	31	32	33	34	35	36
Voltmeter reading	0.09463	0.09129	0.08896	0.08667	0.08456	0.08230
°C	37	38	39	40	41	42
Voltmeter reading	0.08077	0.07820	0.07566	0.07471	0.07252	0.07068
°C	43	44	45	46	47	48
Voltmeter reading	0.06863	0.06671	0.06485	0.06336	0.06213	0.06025
°C	49	50				
Voltmeter reading	0.05920	0.05750				

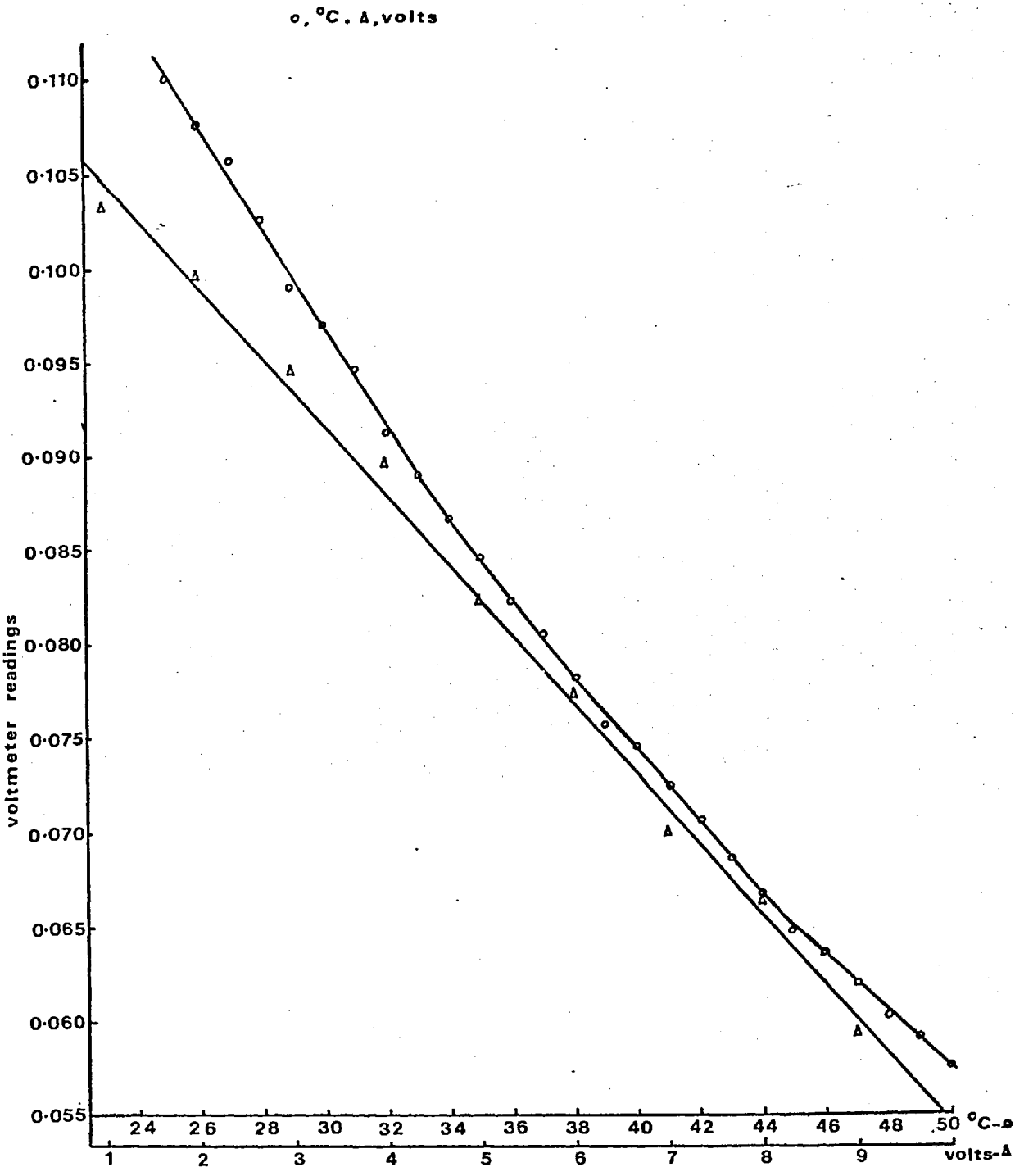
Table 8.2. Relationship between voltage input to the ball, and voltmeter readings from the thermistor

Voltage input	1	2	3	4	5	6	7	8	9
Mean voltmeter reading	0.10334	0.09962	0.09457	0.08988	0.08226	0.07740	0.07011	0.06631	0.05935

Table 8.3. Relationship between voltage input to the ball, and surface temperature

Voltage	1.3	3.4	5.3	7.0	8.5
Temperature ^{o}C	27	32	37	42	47

FIG.8.1. THE RELATIONSHIP BETWEEN VOLTMETER READINGS FROM THE THERMISTOR AND KNOWN TEMPERATURE, OR KNOWN VOLTAGE INPUT, OF THE SURFACE UNDER TEST.



8.2. Method

Two main factors needed consideration in the methods used to investigate probing response to different temperatures. Firstly it was necessary to remove the influence of the prevailing level of locomotor activity from the probing response so that the probing response was measured independently of the tendency to initiate locomotor activity. Secondly if the flies were kept in conditions as near to normal as possible and which allowed normal activity when not being tested, then the consumption of stored nutrients, and any effect the level of these may have on tendency to probe, would have been closer to that encountered by flies under normal laboratory feeding conditions. In practice it was not possible to satisfy both of these requirements.

The first method used was to have the flies free in the usual storage tubes whose plastic lids had been replaced by nylon netting secured by a rubber band. Half of these flies had their wings waxed together to examine how this altered the activity level during recording.

A pyrex domestic casserole dish, floating on a water bath whose temperature could be altered to the required level, was used as the surface of known temperature offered to the flies to see if any probing would be elicited. Each fly was offered each different temperature once each day. Testing began at 27°C, all flies being tested, and then the temperature of the water bath taken up to 32°C, a process which took about 5 minutes, and then all flies tested at this temperature. In this way temperatures of 27°C, 32°C, 37°C, 42°C and 47°C were tested.

Testing began 1 hour after lights-on each day, and was started the first day after the third feed, and was continued for 5 days. Each tube in turn was placed loose netting end down onto the base of the

pyrex dish. If the fly was already touching the dish, a clock was started immediately. If the fly was resting in the top of the tube, a sharp knock was made against the tube to dislodge the fly to the bottom, and then the clock was started. 60 seconds were allowed to each fly. Immediately after starting the clock, the loose netting was gently displaced to one side by 1 mm. This was done to simulate the condition of feeding on a rabbits' ear where friction against the hair due to partial rotation of the tube had been found to encourage probing. The number of flies which probed was recorded.

This method showed that waxing of the wings together did reduce activity in the tubes, and thus the fly remained in contact with the temperature stimulus a greater proportion of the experimental time. However, this did not result in a greater number of probes, and so the 20 flies are treated together in the results. In an attempt to remove the influence of the prevailing level of locomotor activity altogether, and to avoid an increase in humidity as the temperature of the water bath rose, method 2 was adopted, the temperature stimulus being offered in form of the heated polystyrene ball described under materials.

In method 2, flies used were permanently mounted by their dorsal thoraces onto matchsticks, using beeswax. Whilst not being tested, flies had access to a 50 mg polystyrene ball which could be picked up at any time to avoid weakening of the leg muscles, and associated inability to hold the heated ball. The correct height of the fly to enable it to just reach the ball but not use it as a fixed substrate to push against to attempt escape, was maintained by a plasticene bridge across the storage tube.

During testing, after the heated ball had had 5 minutes to reach the required temperature, the matchstick plus fly was held in one hand. The fly was brought down gently on the top of the ball, and then lifted up very slightly so that the fly was holding the ball. Throughout the 90 second recording period the observer altered the angle of the matchstick where necessary so that the fly's walking remained in the plane at 90° to the axis of the gold wire connections to the terminals. As soon as contact between fly and ball was made a stopwatch was started to give a constant recording period.

Each fly was offered each different temperature once for 90 seconds, and the number of seconds to pass before probing were recorded. A priming offer of a ball at 37°C was made ½ hour after lights-on to counteract any post-dawn refractoriness as observed by Brady (1973). After this, testing began at 1 hour after lights-on, when each fly was offered the ball at 27°C, and the results noted. Testing continued at ¾ hour intervals and with 5°C increments, until all flies had been offered each temperature once.

Testing continued from the first day after the third meal until death. This enabled death point, at which flies would have been physiologically very similar to be used for comparison of thresholds of flies to different temperatures, and further comparisons to be made between flies at similar distances from death. It was considered that comparisons made this way could be more meaningful than comparisons made from the time of food intake forwards.

8.3. Results

The results of the first method are given as Table 8.4. and Fig.8.2. and it can be seen that at 27°C, only just above the ambient temperature, fewer flies probed than at the higher temperatures. For the other temperatures, on day 1 there were few flies probing. However, by day 2, many flies were probing at 37°C, and there is a clear difference between the numbers of probes induced by each temperature. Over the next three days, the range of response narrows whilst the numbers probing increases, so that by day 5, with the exception of 27°C, all temperatures were eliciting close to 100% probing response.

Table 8.4. Percentage of 20 flies probing at different temperatures on different days

Temperature °C	Days since last meal				
	1	2	3	4	5
27	5	5	20	35	35
32	30	45	75	90	95
37	45	85	90	95	100
42	25	80	90	90	100
47	20	55	85	95	100

The results of the second method are given as Tables 8.5. and 8.6. and Figs 8.3. and 8.4. Under these experimental conditions flies did not usually begin to probe until the third or fourth day after the last blood meal, so that the fourth day before death is usually the fourth day after the meal.

FIG. 8.2. THE EFFECT OF A RANGE OF TEMPERATURES ON A GLASS SURFACE ON THE NUMBER OF FLIES PROBING EACH DAY

Ordinate: Percentage of flies which probed

Temperatures: ▲, 27°C. ○, 32°C. ▲, 37°C. ●, 42°C. □, 47°C.

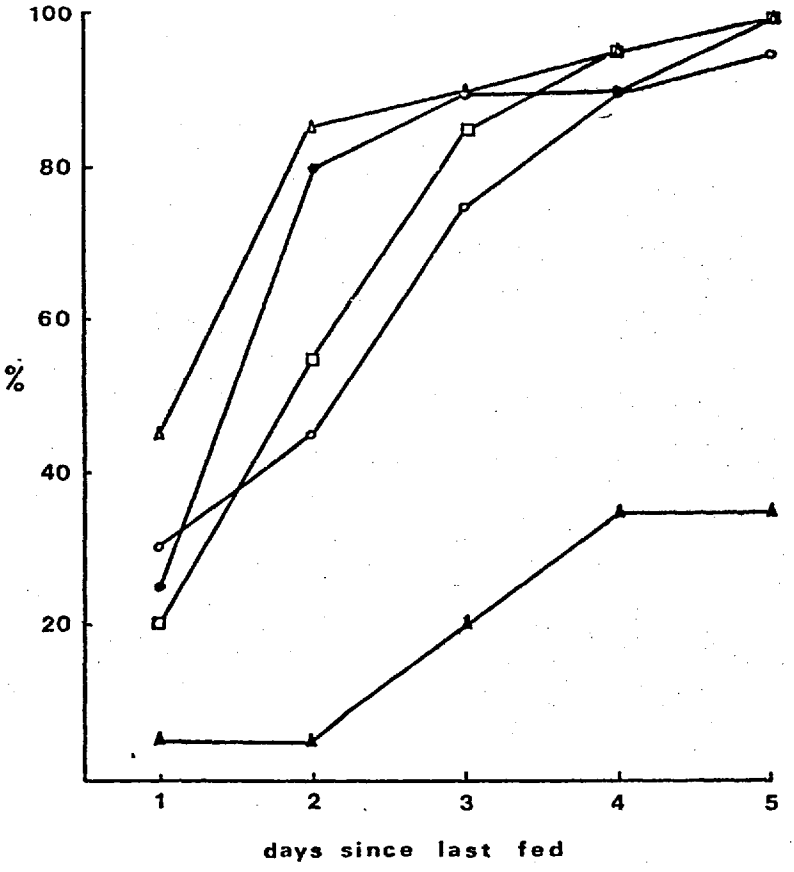


Table 8.5. Percentage of 15 flies probing at each temperature on different days before death

Temperature °C	Days before death			
	4	3	2	1
27	7	40	67	67
32	13	40	67	100
37	0	40	87	100
42	0	40	67	100
47	7	27	60	100

Table 8.5. and Fig.8.3. for percentage response per day show similar trends to those shown by the first method. There is an increase in responsiveness to all temperatures with starvation, with 27°C eliciting fewest probes and 42°C and 37°C eliciting the most probes. At an early stage in starvation, on day 4 before death, there is a similar response to all temperatures, with the range of response widening up to the second day before death, and then narrowing sharply on the last day before death.

In order to remove the buffering effect of the many 90 second no probe entries from any difference in response times at different temperatures during the early days when fewer flies were responding, mean response times were calculated excluding flies which had not probed. These are given in Table 8.6. and Fig. 8.4. and a similar picture of the changes in threshold with the approach of death emerges. On the third day before death few flies probed (4 at 47°C and 6 at the other temperatures) giving no clear pattern, but on the second day before death, when at least 9 flies were probing at each temperature, there is a wide range of response, with a difference of 15.5 seconds

FIG. 8.3. THE EFFECT OF A RANGE OF TEMPERATURES IN THE POLYSTYRENE BALL ON THE NUMBER OF FLIES PROBING EACH DAY

Ordinate: percentage of flies which probed
Temperatures: solid lines—▲, 37°C. □, 47°C.
: broken lines—▲, 27°C. ○, 32°C. ●, 42°C.

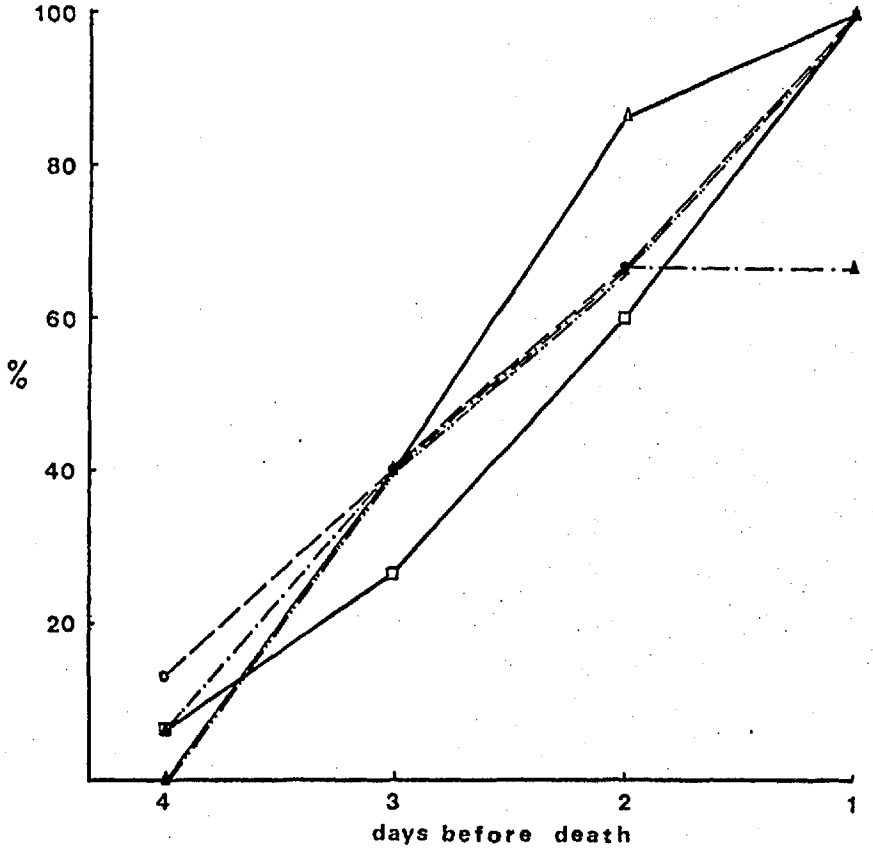
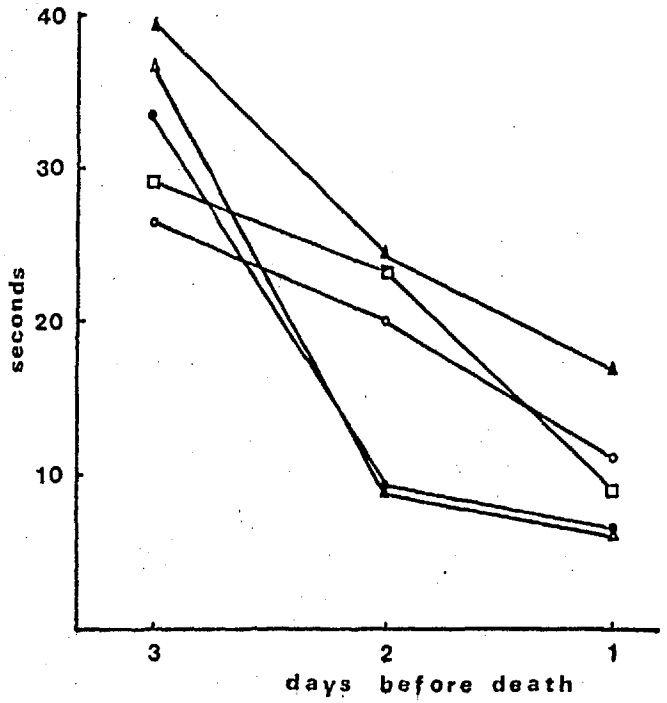


FIG. 8.4. THE EFFECT OF A RANGE OF TEMPERATURES IN THE POLYSTYRENE BALL ON THE MEAN LENGTH OF TIME TAKEN TO PROBE EACH DAY

Temperatures: ▲, 27°C. ○, 32°C. ▲, 37°C. ●, 42°C. □, 47°C.



between the fastest and slowest mean times to respond. The preferred temperatures are again 37°C and 42°C. By the last day before death, there is only a range of 7.5 seconds in between the fastest and slowest mean times to respond. Thus, during late starvation, temperatures of 47, 32 and 27°C, which were least preferred at an early stage in starvation become increasingly acceptable.

Table 8.6. Mean number of seconds to pass before probing at different temperatures on different days ± S.E.

Temperature °C	Days before death		
	3	2	1
27	39.4±11.3	24.4±5.6	13.9±2.7
32	26.4± 7.1	20.2±5.8	11.3±6.4
37	36.9±13.4	8.9±2.1	6.4±1.5
42	33.5±11.1	9.1±1.6	6.8±1.2
47	29.0± 7.7	23.3±8.2	9.1±3.4

In the second method flies mounted onto matchsticks by beeswax and with access to a light polystyrene ball to hold lived a mean 7.4 days. This is considerably longer than similar mature flies used by Brady (1973) which were mounted by Uhu glue, and had no access to a ball to hold. 60% of these flies had died by the end of day 4.

On day 1 before death, minimum mean response times of 6.4 and 6.8 seconds were recorded from 37°C and 42°C respectively. Only ca.7% of the responses at the optimum temperatures occurred within 2 seconds, whereas Brady (1973) recorded that ca.20% of responses on day 4 after the meal occurred within

2 seconds. The greater minimum response time may be explained by the greater weight of the polystyrene ball at 290 mg, nearly 5 times heavier than the foam rubber ball used by Brady.

8.4. Discussion

There is a change in temperature acceptance threshold with starvation which results in a wider range of temperatures eliciting probing. Those temperatures least preferred early in starvation apparently show a greater rate of increase in acceptability across starvation than optimum temperatures. This must be explained by some of the flies at optimum temperature reaching a minimum response time early on in starvation, and thus those flies would add a constant response time to the means of times on later days, buffering the decrease in response times of other flies in the optimum groups. At least-preferred temperatures, since minimum response time possible at more preferred temperatures were only approached on day 1 before death (and then not closely at 27°C), all flies were capable of a daily decrease in response time, giving an apparently greater rate of decrease.

Whilst the temperature variation is the sensory input most directly applied to the flies in this experimental situation, other factors may have influenced the response. Dethier (1954) detected no effects of odour on probing response, and Hughes (1957) only recorded an increase in probing when high concentrations of volatile substances were used. However, Mitchell and Reinouts van Haga-Kelker (1976) showed that flies used to feeding on rabbits showed an improved feeding response if rabbit odour was present during a meal fed through an artificial membrane system, so that odour at least acts as an indicator of a familiar feeding

situation to flies. Olfactory inputs were not controlled, but should have been identical at each recording, and since flies were not fed after recording began, there is no reason to expect that the seasory input from the human odour present would have altered across starvation.

Probing responsiveness continues to increase until the last day of life, whereas spontaneous locomotor activity reaches a peak three days before death (see Chapter 7.), and declines thereafter. It is unknown if the reduced spontaneous locomotor activity is due to a shortage of nutrient, or a rise in threshold. In the latter case, the two acts of flight and probing would be under different mechanisms of control. Barton Brown and Evans (1960) concluded that in the blowfly feeding response and locomotor activity thresholds were not controlled by the same mechanism.

CHAPTER 9

9. RECORDING OF OBSERVED SEQUENCES OF BEHAVIOUR TO SHOW ANY CHANGE ACROSS STARVATION

9.1. Apparatus

9.1.1. Actograph

An actograph the same as the larger of those described in section 3.1.1. was used. It was housed in a 26.7 cm x 12.1 cm x 10.1 cm box, with the two narrow side walls of ply-wood painted white. The base of the box was black ply-wood. The two side walls had a 5 mm groove milled 6 mm from the front. Into this slot was placed a 25.4 cm x 25.4 cm one-way mirror. The remaining longitudinal wall was made of clear perspex attached between the dexion frame corners and the side walls. Fixed to the one-way mirror by corner attachments points was a screen of white card to prevent any outside visual stimulation. A 2.5 cm x 10.1 cm aperture was cut into the front of the screen to allow observation. A 30 cm diameter kymograph drum covered with white card except for a 12 mm wide vertical black stripe was placed so that the drum was in the centre of the clear perspex back wall of the chamber; the nearest point was 1.3 cm away from the perspex. Wings of white card were positioned so that except for when the black stripe was passing, only white would be visible from inside the box. The visibility of the black stripe from within the box was tested. A small hand mirror was held inside the wings and just behind the clear rear wall, facing towards the one-way mirror at the front of the box. This established that when the drum was revolving, the stripe was visible in the one-way mirror as well as as a real image.

The roof of the box was of 5 mm opal perspex, which fitted securely between the one-way mirror and the dexion frame. The box was

illuminated by a 5 watt Mazda white fluorescent tube attached to the dexion frame. The light was operated via a time clock and gave a light intensity in the box of 1100 lx.

An odour-free air supply was provided. A fish tank pump passed air through activated charcoal in a 5.1 cm deep x 5.1 cm diameter sealed container. A tube from this vessel fed into a 5.1 cm diameter plastic beaker glued onto a side wall and surrounding a number of air holes drilled through the wood.

9.1.2. Arena

Fig.9.1. shows the arena used. The volume of this was ca.890 litres, some 3,100X that of the actograph.

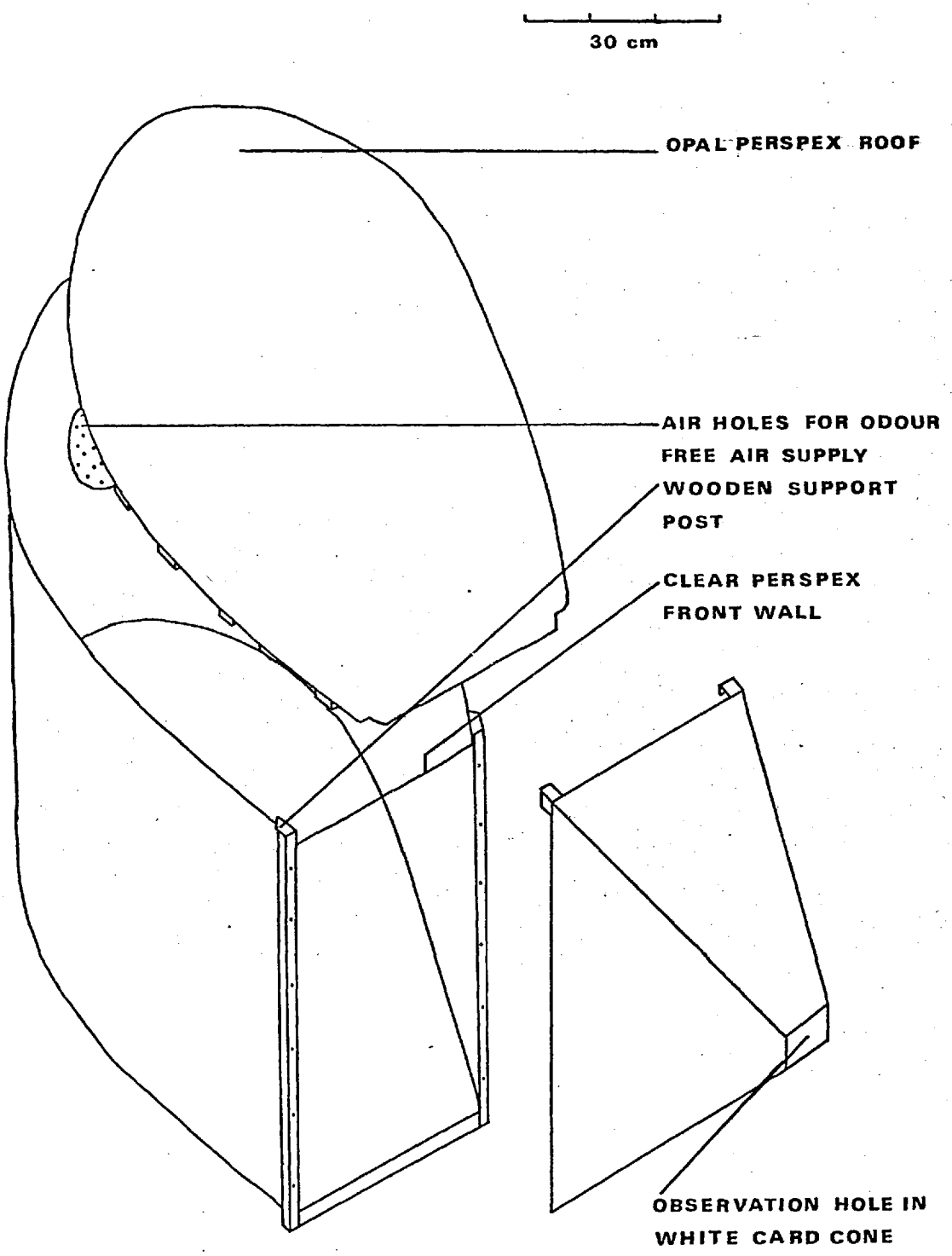
The base of the arena was made of 1.3 cm chipboard and the sides of ply-wood bent round it. The structure was made rigid by 2 1.3 cm square wooden posts glued to the front outside of each wall and planed flat so that the clear 6 mm perspex front could be screwed in position, thus holding the sides firm. The roof was of 6 mm opal perspex overlapping the walls, and was prevented from moving by wedges of perspex cemented to the perimeter under surface.

A circular pattern of air holes covering an area with a diameter of 24 cm was made in the rear curved wall. A polythene chamber was sealed onto this, and odour free air was pumped into the chamber as described above.

A cone of white card with a black card floor was supported by the wooden posts at the front of the arena, so concealing the room from the fly inside the arena. A 5 cm x 8 cm aperture was made to allow observation.

The entire inside of the arena was covered with nylon netting of the same grid size as that used to make the actographs, to imitate

FIG.9.1. DIAGRAM OF THE ARENA USED FOR OBSERVING SPONTANEOUS ACTIVITY.



the surface pattern. The netting was attached by Durofix glue to the floor and walls, and by the action of chloroform drops to the perspex roof and front.

The arena was illuminated by 2 40 watt Phillips White fluorescent lights. These were suspended above the arena at a height that gave light intensities as measured by a Selenium photocell of 800 lx. at the bottom, 1200 lx. at half height, and 2800 lx. immediately beneath the opal perspex roof.

9.2. Glossary of terms used to describe behaviour

The following lists the terms used to denote different forms of activity. For a full description of the format and duration of the activities, see Results sections.

Flies would remain immobile for relatively long periods (mean all flies in the actograph, day 1, 7 minutes) during which there was no visible activity. Such periods of rest were interspersed with shorter periods (ca. 2 minutes) of intense activity. Such activity is described as occurring in a 'burst' (different 'burst' types described below). The long periods of rest between 'bursts' are described as 'inter-burst gaps'.

Within a burst no two activities were ever observed to occur at the same time. Each activity was recorded separately for the duration it was being performed. A single continuous performance of any act within a burst is described as a 'bout'. A series of 'flight bouts' alternating with 'rest bouts', and less frequently interspersed with 'cleaning bouts' or 'walking bouts' (the sequence lasting ca. 2 minutes), is described as an 'activity burst'. Apart from the 'cleaning bouts' which occurred infrequently throughout, such 'activity bursts' would frequently be initiated and ended by a bout of cleaning, called pre-burst and post-burst cleaning respectively. During analysis, when reference is made to the length of an 'activity burst' minus the length of such cleans, it is described as the length of a 'truncated activity burst'.

A series of cleaning motions, not interspersed with any form of locomotion is described as a 'cleaning burst'.

Where the term 'arousal' is used in an attempt to partially explain some aspect of tsetse fly behaviour as reported in this thesis, it is used in Andrew's (1974) responsiveness sense of the word.

9.3. Methods

9.3.1. Recording

Mature male flies were used throughout the observations. The behaviours recorded were walking, resting, cleaning, flight and probing. Cleaning was treated as a single act and was not broken down into constituent parts. Flies were placed in the actograph or the arena shortly after their third meal. Recording was begun the following morning, and continued for 5 days, or until the level of activity had declined as a prelude to death. The actograph and arena used are described above (Apparatus).

A paper tape punch for 8-track tape was used to make a binary record of the behaviour. Early records were made via a toggle switch interface which required that each toggle had to be switched off and a new channel opened when behaviour changed. Later records were made via a push button interface whereby the depression of one button automatically cancelled the previous depression, except in the case of one master switch which, when opened, added a further row of punch holes to the ongoing line. Each behaviour was allocated a code hole, whilst other information such as total time, or presence of the black stripe used for visual stimulation, was recorded by use of the master switch. The paper tape was set to emerge at 3 holes per second, so that rapid changes in behaviour could be recorded. During all recordings, the tape punch was housed in a separate room from that in which the observations were made, in order to minimise any auditory or vibrationary input.

Each morning, the paper tape punch was set into action shortly before the lights were due to switch on. Recordings of spontaneous activity in the actograph or in the arena were begun at lights-on, and continued for $1\frac{1}{2}$ hours. This would have recorded at least 40% of that day's flight activity (Brady and Crump, 1978). Recordings from the actograph, when spontaneous bursts of activity were alternated with stimulated bursts, were begun at lights-on, and continued until the required number of bursts had been recorded, (see below).

For recording of spontaneous activity in both the actograph and the arena, care was taken that virtually constant environmental conditions were maintained throughout the recording period.

For recording of spontaneous bursts alternated with stimulated bursts of activity, the first two spontaneous activity bursts of the day were recorded undisturbed as usual. Preliminary analysis of the length of gaps within bursts from the spontaneous activity recordings had shown them to be less than 10 seconds in length. During the second spontaneous burst, as soon as 10 seconds had passed with no activity, and the second burst of the day had thus ended, the kymograph drum was switched on. The kymograph was always stopped with the black stripe just about to become visible, and so the stripe usually became visible within 1-2 seconds of restarting the kymograph.

The apparent angular velocity of the stripe from the centre of the actograph was 14.3°/second, with an angular width of 26.7 cm. The stripe was visible for ca. 7 seconds, each complete revolution lasting 17 seconds. Preliminary testing had shown that at least early in the hunger cycle, six passages of the stripe across the field of vision were often enough to induce habituation (i.e. for the flies to have ceased to respond). In a previous investigation of tsetse visual responsiveness, Brady (1972b) had found the majority of flights, in response to a stripe moving at an angular velocity of 13.5°/second to occur within the first 12 traverses of the stripe and within 60 seconds.

As soon as the black stripe was visible to the observer, the master switch on the interface to the paper tape punch was pressed and was left down until the stripe had just disappeared for the 6th time. In this way, 6 periods of stripe visible, and 5 of stripe invisible were recorded, lasting 93 seconds in total. The time of the last disappearance of the stripe was noted. The third spontaneous activity burst was then waited for, and recorded as usual.

The presentation of the stripe within a few seconds of the second burst of spontaneous activity ending meant that the stimulus had been offered very close to a period of high probability of a spontaneous burst occurring. In an attempt to give the visual stimulation at a time of low probability of a spontaneous activity burst occurring, the interval between the last stripe of the stimulation after the second spontaneous burst and the first act of the third spontaneous burst was noted. The third spontaneous activity burst was allowed to end naturally, and the time of ending was noted. $\frac{1}{3}$rd of the interval time noted above was then allowed to elapse, so that a time of minimum probability of an activity burst should have been reached. The visual stimulus was then offered again, as described above.

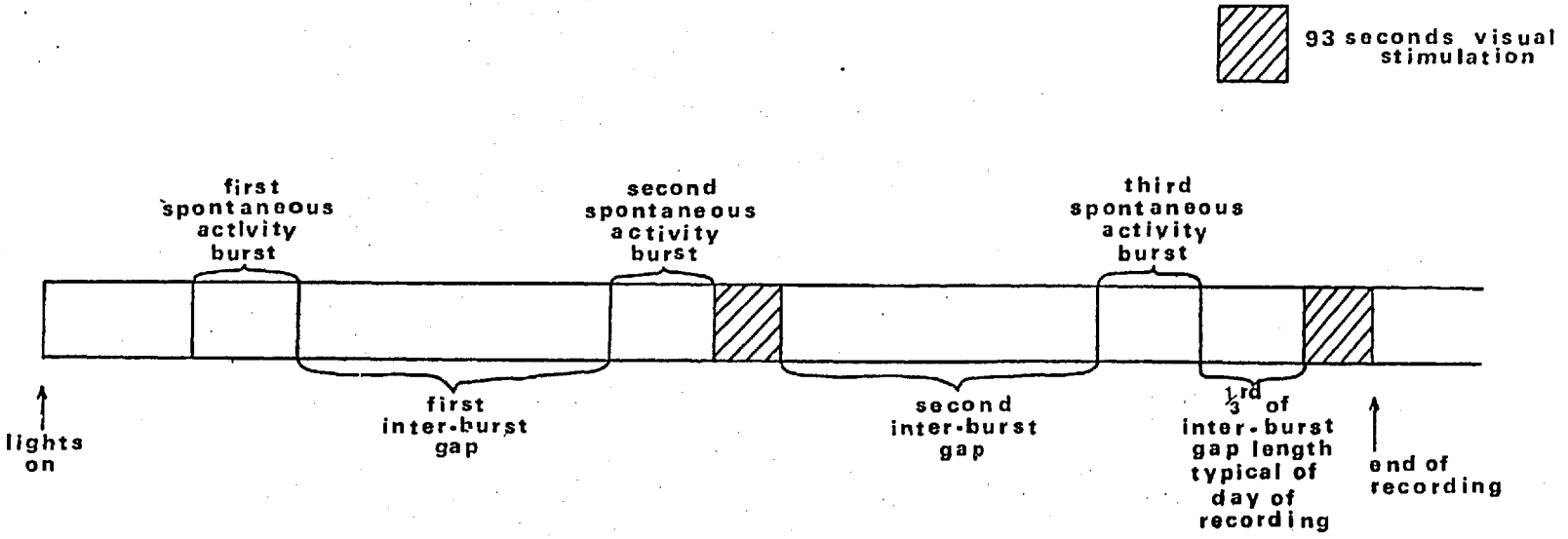
Fig.9.2. illustrates a typical sequence of spontaneous bursts alternated with stimulated bursts.

At the end of each recording session, the tape was clearly marked with experiment number, fly number and day, and stored pending analysis.

9.3.2. Analysis of the paper tape

Analysis of spontaneous activity in the actograph and the arena, was as follows. Programmes written by A.R. Ludlow were used to condense the raw tape data into a series of code letters indicating

FIG.9.2. SEQUENCE OF SPONTANEOUS BURSTS OF ACTIVITY ALTERNATED WITH PERIODS OF VISUAL STIMULATION



the behaviour, each with its respective length in seconds. This list was printed out and stored for further analysis.

The sequence of different activities was then divided into bursts of activity. 20 seconds was used as the length of rest to be exceeded to qualify as an inter-burst gap. This length left a safety margin outside the usual within-burst gap of under 10 seconds, so that there was no danger of analysing the data into too many bursts, with within-burst short gaps incorrectly labelled as between-burst gaps. By contrast, between-burst gaps were typically much longer than 20 seconds, so that there was no danger of a between-burst gap being 'missed'. The programme then printed out a table of the number of bursts, the frequency and duration of the behaviours constituting them, and the inter-burst gap length. A programme written by the author worked out the mean bout length for each activity in each burst (see Appendix II). For analysis of spontaneous bursts of activity alternated with stimulated bursts, the programme by A.R. Ludlow was used to condense the data as before except with double the number of codes, since each behaviour now occurred in two different situations: spontaneous, or during visual stimulation. Further analysis was by a programme written by the author, as follows.

The data were analysed into activity bursts, again using 20 seconds as the gap to be exceeded to indicate an inter-burst gap. Bursts and gaps which occurred spontaneously were distinguished from any time when the black stripe was present. Any acts occurring within the period of stimulation were further segregated into those which occurred whilst the stripe was visible, with the numbers and lengths of each behaviour in each burst stored in a two-dimensional array; and those which occurred when the stripe was invisible, stored in a further two-dimensional array. This analysis into activity bursts under the 3 different circumstances was printed out. Where activity continued after visual stimulation had ceased, without a gap of longer than 20 seconds, a new burst of spontaneous activity was registered, since

the extra punch hole line indicating visual stimulation was no longer present. The very short inter-burst gap of one or two seconds introduced the activity which followed as being post-visual stimulation, i.e. not a separate unstimulated burst of spontaneous activity which would have required a gap of greater than 20 seconds. Recording in this way gave a measure of the length of time, after visual stimulation had ceased, for which the fly remained active. Appendix II shows the program written by the author to analyse the spontaneous/stimulated data after preliminary reading of the paper tape. In addition to analysis into bursts, the program worked out the mean length of each activity bout in each burst in the same way as these measures were calculated for tapes of spontaneous activity.

9.4. Presentation of Results

With the appreciation that drive concepts are of limited usefulness (Hinde, 1970) the value of tracing the trends of different acts separately to aid evaluation of associations between those acts has been realised. Data such as these presented here will be non-stationary, i.e., the probabilistic structure of the system will change in time both across the 1½ hours as part of the circadian rhythm changes recorded by Brady, (1972a), and across starvation (Brady, 1975). The rates of change will vary between flies and thus the balance between different behaviours across starvation will alter at different rates in different flies. Amalgamation of data from different flies to examine changes across starvation would thus obscure any underlying relationships. The data from each fly are therefore presented separately for examination of the balance between acts across starvation. For comparison of gross trends, such as the differences between behaviour in the actograph or arena, means from all flies are used as usual.

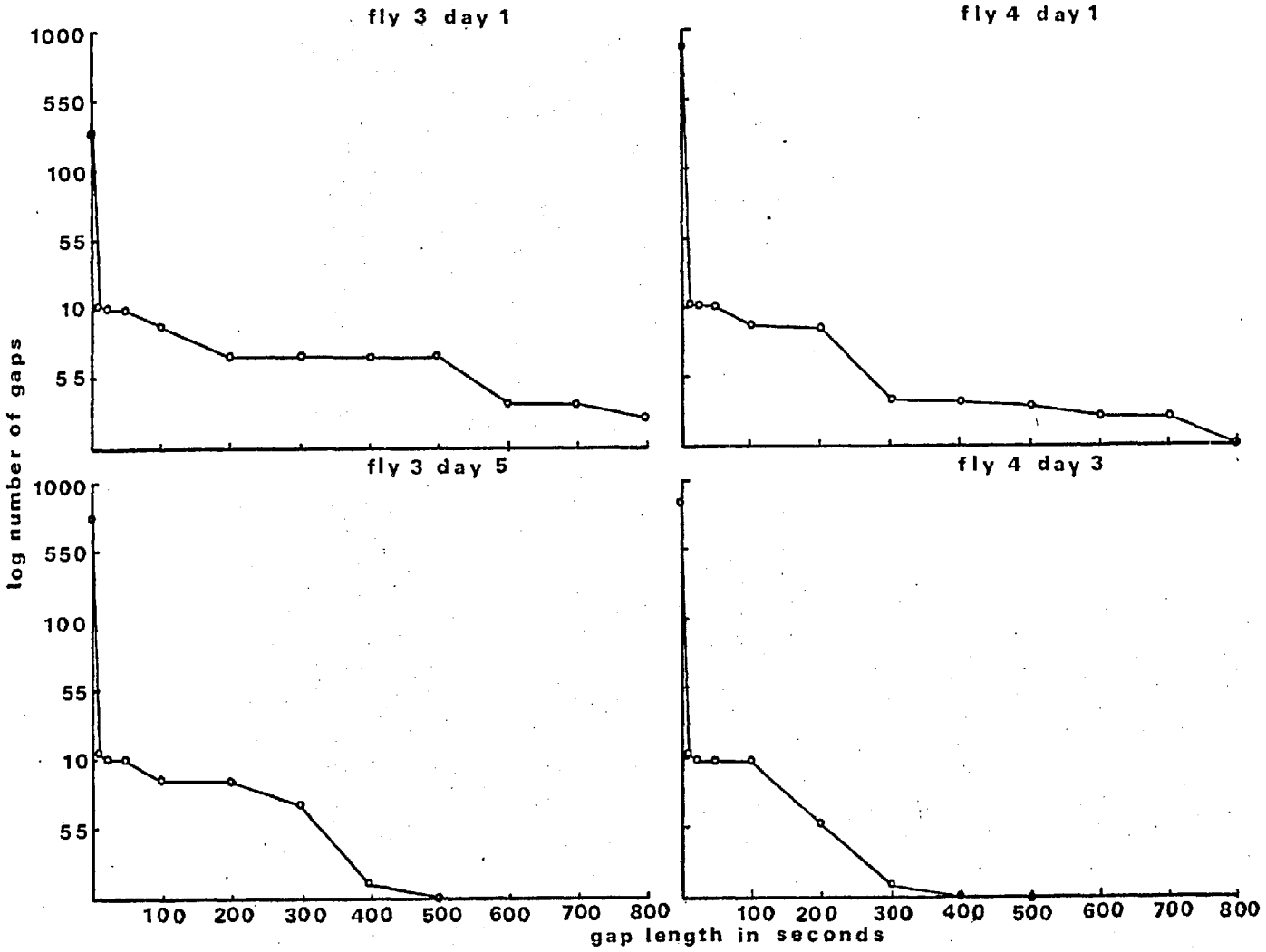
9.5. Results and discussion of Spontaneous Activity in the Actograph

9.5.1. Log survivor functions

During analysis of sequences of behavioural events, the log number of events whose duration exceeded various increments across the range of length exhibited, plotted against the length increments, gives a log survivor function. This has been used by Slater to split behaviour into bouts and gaps, e.g. with the preening behaviour of Zebra finches (Slater, 1974a), and with the feeding patterns of Zebra finches (Slater, 1974b). The slope of the plot is proportional to the probability of an event with the passage of time since the last event. Fig 9.3. shows the log survivor functions for all gaps or periods of rest (i.e. inter-burst gaps, and within-burst rest bouts) for a number of different flies. The point at which there is a sudden reduction in the probability of a gap of that length occurring can be used to differentiate between gaps occurring within a burst, and those occurring between bursts. Fig.9.3. shows that the transition point from intra-burst to inter-burst gaps occurs close to 10 seconds.

The survivor functions also show that the slope is not constant beyond the transition point, as would be expected if the gap lengths were randomly distributed and there was an equal probability of any gap length occurring. Where there is an increase in slope (e.g. between 500 and 600 seconds for Fly 3 day 1, and 300-400 seconds for Fly 3 day 5), then the probability of a gap continuing is decreased and there is thus an increased probability of a new burst of activity at that point in time. Gap lengths cluster around the time length just prior to the change in slope, and this length is shorter on day 5 than on day 1 for fly 3, i.e. it decreases with an increase in number of bursts. Brady (1972a; 1975) had shown that flight burst length measured in actographs remained constant across starvation, and that the change in activity level was achieved by

FIG.9.3. LOG SURVIVOR FUNCTIONS FOR GAPS.



modulation of the periods of inactivity. Fig.9.8. (triangles) shows the pattern of change of mean inter-burst gap length across successive days of starvation, e.g. Fly 3 shows a decrease in inter-burst gap length from ca. 370 seconds on day 1 to ca. 210 seconds on day 4.

9.5.2. Activity Bursts

Typically an activity burst would consist of 20-30 bouts of flight of mean length ca. 2 seconds alternating with bouts of rest (during which no activity was visible) of mean length ca.2 seconds infrequently interspersed with bouts of walk of ca. 1 second and bouts of clean varying between 2-10 seconds in length. The mean length of bouts of individual behaviours each day is shown in Fig.9.5. and Fig.9.8. Pre-burst and post-burst cleans gave mean lengths varying between 5 and 60 seconds, i.e. they tended to be longer than within-burst bouts of clean. The recording taken of a typical activity burst is shown in Fig.9.4.

The mean lengths of activity bursts each day are shown in Fig.9.6. Two different measures of length are given. The complete activity burst length, i.e. that including any pre-burst and post-burst cleaning is given and its mean for all flies was 133 seconds. The truncated length, i.e. excluding pre-burst and post-burst cleans, is also given. This is the length that would be measured if movement of an actograph due to flight was used to monitor the length. The mean of this for all flies was 110 seconds. This is more than double the mean length of flight burst of 45 seconds given by Brady (1972a) and the mean length of 47 seconds given in this thesis (Table 3.1.). Both the shorter means were recorded in actographs, the latter in identical ones to that used for these observations.

Fig.9.4. Typical Activity Burst

* Fly 3 Activity Burst Number 11 Day 2.

Key: A Clean. B Walk. D Flight. E Rest.

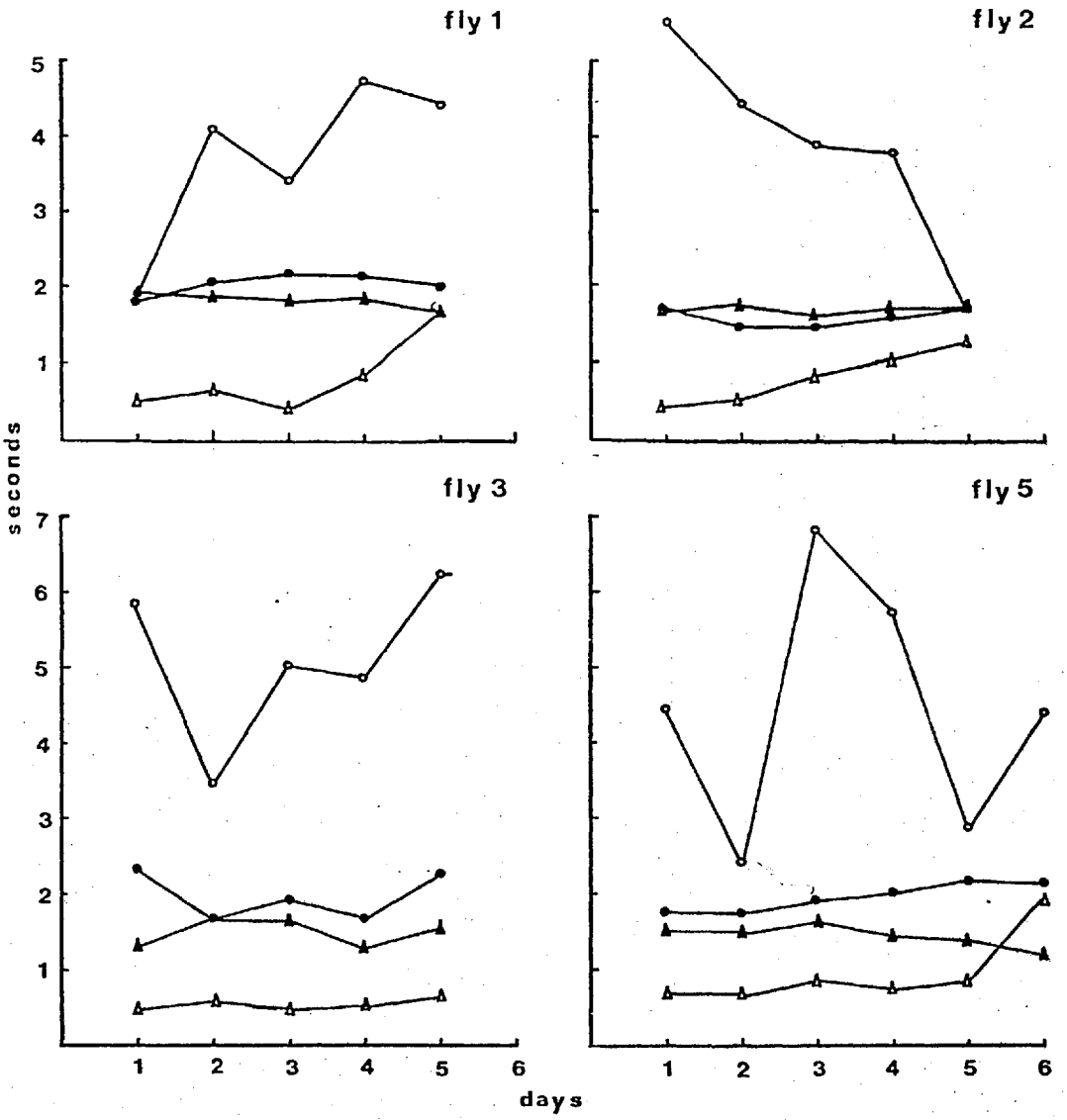
732.7E	1.3D	.7E	2.0D	3.0E	2.3D	2.3E	.7D
1.0E	.3B	1.0E	.3B	1.0E	3.7D	5.3E	1.7A
.7E	3.3D	.7E	1.0D	2.7E	1.7D	2.7E	1.0D
1.0E	1.3B	2.7D	1.0E	1.0D	1.7E	1.3D	.7E
1.3D	2.3E	1.7D	2.0E	2.0D	4.0E	1.0D	.3E
1.7D	3.0E	8.0A	.3E	2.0D	3.0E	2.0D	.3E
1.0D	.7E	.7B	2.0E	1.3D	2.3E	.7D	1.0E
1.0D	2.0E	1.7D	2.3E	1.0D	1.3E	.3B	1.7E
1.0D	.3E	1.0D	1.0E	2.0D	9.0E	1.3D	3.0E
13.0A	1102.0E						

Analysis of Activity Burst

Burst Number	Bouts of Cleaning		Bouts of Walking		Bouts of Flight		Bouts of Resting		Length	Gap
	Length	Number	Length	Number	Length	Number	Length	Number		
11	22.7	3	2.7	4	45.7	29	66.3	34	137.3	1102.0

FIG. 9.5. MEAN LENGTH OF BOUTS OF DIFFERENT ACTS EACH DAY

o, clean ●, rest Δ, walk ▲, flight



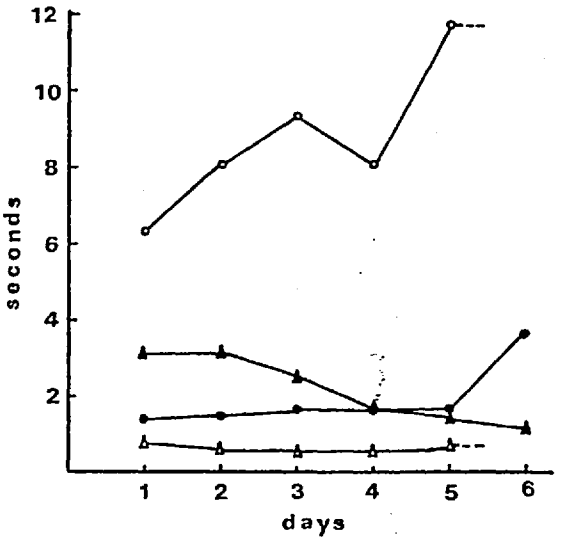


FIG. 9.5. Cont^{d}.

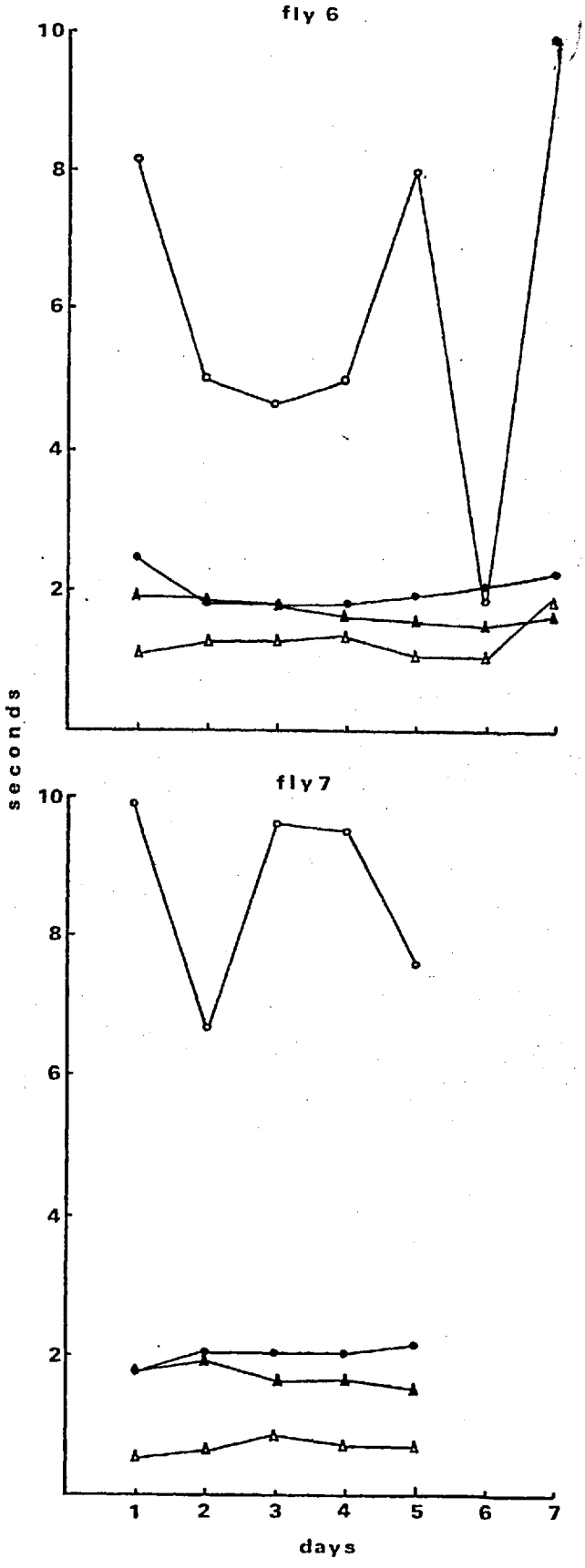


FIG. 9.6. MEAN LENGTH OF BURSTS EACH DAY

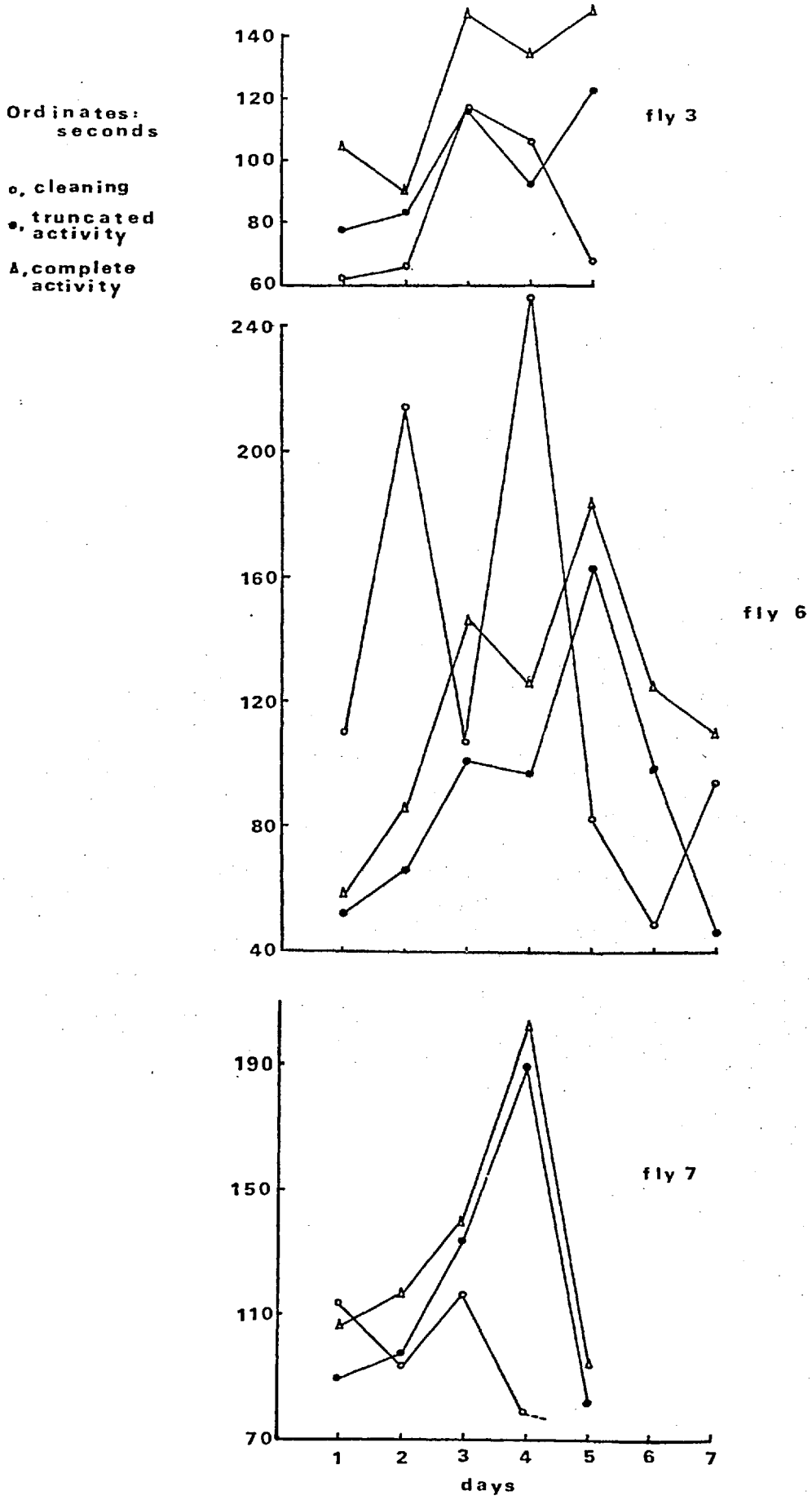
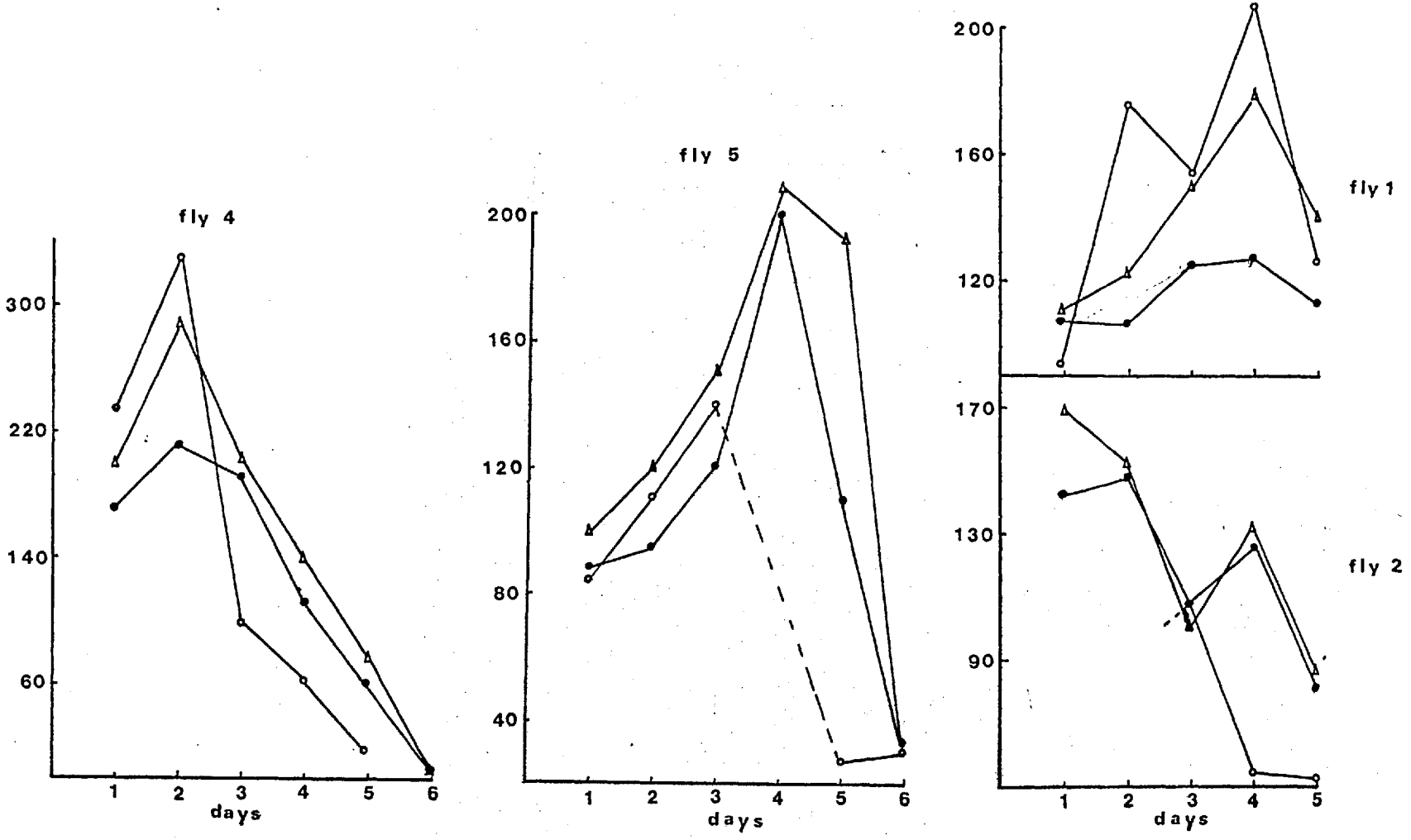


FIG.9.6. Cont^{d}.


fly 4
300
220
140
60
1
2
3
4
5
6
days
fly 5
200
160
120
80
40
1
2
3
4
5
6
days
200
160
120
fly 1
170
130
90
fly 2
1
2
3
4
5
days

Intra-burst bouts of clean were so short (see above) that any break in actograph rocking would not be long enough to be identified as an inter-burst gap on the Edgecumbe Peebles paper output from the actographs. The difference between length of activity bursts must reflect both the latency between the onset of flight and the rocking of the actograph (the box rocked due to a landing and rest rather than to flight itself) and the recording lost due to the fly remaining at one end either immediately before the end of a burst, or at the very beginning.

9.5.3. Cleaning activity

The second type of burst consisted only of cleaning with no locomotion. Fig.9.6. shows the mean lengths exhibited range from 35 - 330 seconds. The lack of locomotion may indicate that a cleaning burst had different causal factors than an activity burst.

For recording on tape, only the presence or absence of clean was noted, but notes were made of the areas cleaned. There were three types of cleaning burst. In 'front cleans', the forelegs were used to groom the mesothorax, prothorax and proboscis, and to rub against each other. In 'rear cleans' the hind legs were used to clean the wings and abdomen. The third type exhibited both front and rear cleans, with time equally divided between the two, and the transition always being from front to rear, never in reverse. Dawkins and Dawkins (1976), in a study of grooming in blowflies, found that the commonest transitions between different acts of grooming occurred between acts with the fewest differences in parts of the body involved. They placed this phenomenon under the rubric of 'postural facilitation'. However, this cannot explain the observed one-way transition here, since flies could go into rest directly from both types of clean, and go into both types of clean directly from rest. In

addition, there must be as many differences front to rear clean as rear to front, so that transition one way should be as 'expensive' in terms of posture changes as transition the other way. Instead, it might be assumed that rear cleaning was so effective at raising the threshold to clean that it was never followed by front clean, or that at lowest thresholds front clean was always favoured which then left the option of a rear clean once the threshold had risen a little. Alternatively, it is possible that the transition rear to front clean was not a possible route on the nervous network. In this case, the 'decision', (as used by Dawkins and Dawkins, 1973) to go into rear cleaning is the end of that sequence of decisions, with possible filtering out of any cleaning stimuli subsequent to a rear clean. By contrast, the decision to go into a front clean is followed by a further decision to stop cleaning, or to go into rear clean.

The short hurried bouts of clean which occurred within activity bursts, and pre- and post-burst cleans were always front cleans, and this may be explained by postural facilitation if for take off the rear legs must be in contact with the substrate, as they are in front cleans. It is not possible to say from the data whether these cleans are displacement acts, disinhibited due to some frustration of the flight tendency, or whether they occur as part of a chained sequence of different events, i.e. due to an increase in irritation resulting from flight in a confined space.

There are thus three different contexts of cleaning: complete cleaning bursts isolated from locomotion; pre-burst and post-burst cleans being interrupted by, or ending activity bursts; and hurried short bouts of clean always of the front, which occurred within the activity burst.

9.5.4. Preliminary examination of the balance between activity and cleaning bursts across starvation

The data were examined for change in performance of cleaning and activity bursts across starvation. Fig.9.7. shows that the number of activity bursts was at a low level on day 1, rising to a clear peak in 1 fly on day 2, 1 fly each on days 3 and 4, and 2 flies on day 5. Flies 1 and 6 did not show a clear trend. Previous published work (Brady, 1975) had shown the mean number of daily flight bursts (as recorded by an Edgecumbe Peebles recorder), from a large number of mature flies to increase up to day 5. The number of cleaning bursts did not show such a clear trend. Only in fly 2 was there a steady increase in numbers across starvation.

In fly 2, numbers of cleaning and numbers of activity bursts both reach a peak on day 5. In flies 3 and 7 the numbers of cleaning bursts reached a peak after the peak of activity bursts and in fly 4 before the peak of activity bursts. The performance of each burst type does not always appear to be independent, e.g. in fly 1 the increase in the number of cleans on day 3 appears to be at the expense of the number of activity bursts, in fly 3, the number of cleans is suppressed on days 2, 3 and 4 whilst activity bursts increase and only increases again once the number of activity bursts begins to decline. Again, fly 6 shows opposite trends for the two burst types on days 3 and 5.

The performance of one burst may influence the performance of the other via competition for 'performance time', when this quantity is determined before allocation of it to the different acts. Different burst types may also appear to influence each other where conditions favouring the performance of one do not favour performance of the other. Such a condition might be the level of arousal, if the probability of different responses occurring varied with changes from 'sleep' to 'wakefulness' in an 'activity cycle', with short term fluctuations in probability reflecting these changes (Andrew 1974). Such a cycle may perhaps be extended to account for the long term changes in different responses observed across days of starvation. However, steady progress through such a cycle would be expected to yield two approximately straight lines of

FIG. 9.7. THE DISTRIBUTION OF CLEANING AND ACTIVITY BURSTS ACROSS STARVATION

Ordinates: number, abscissae: days
Bursts: o, cleaning Δ, activity

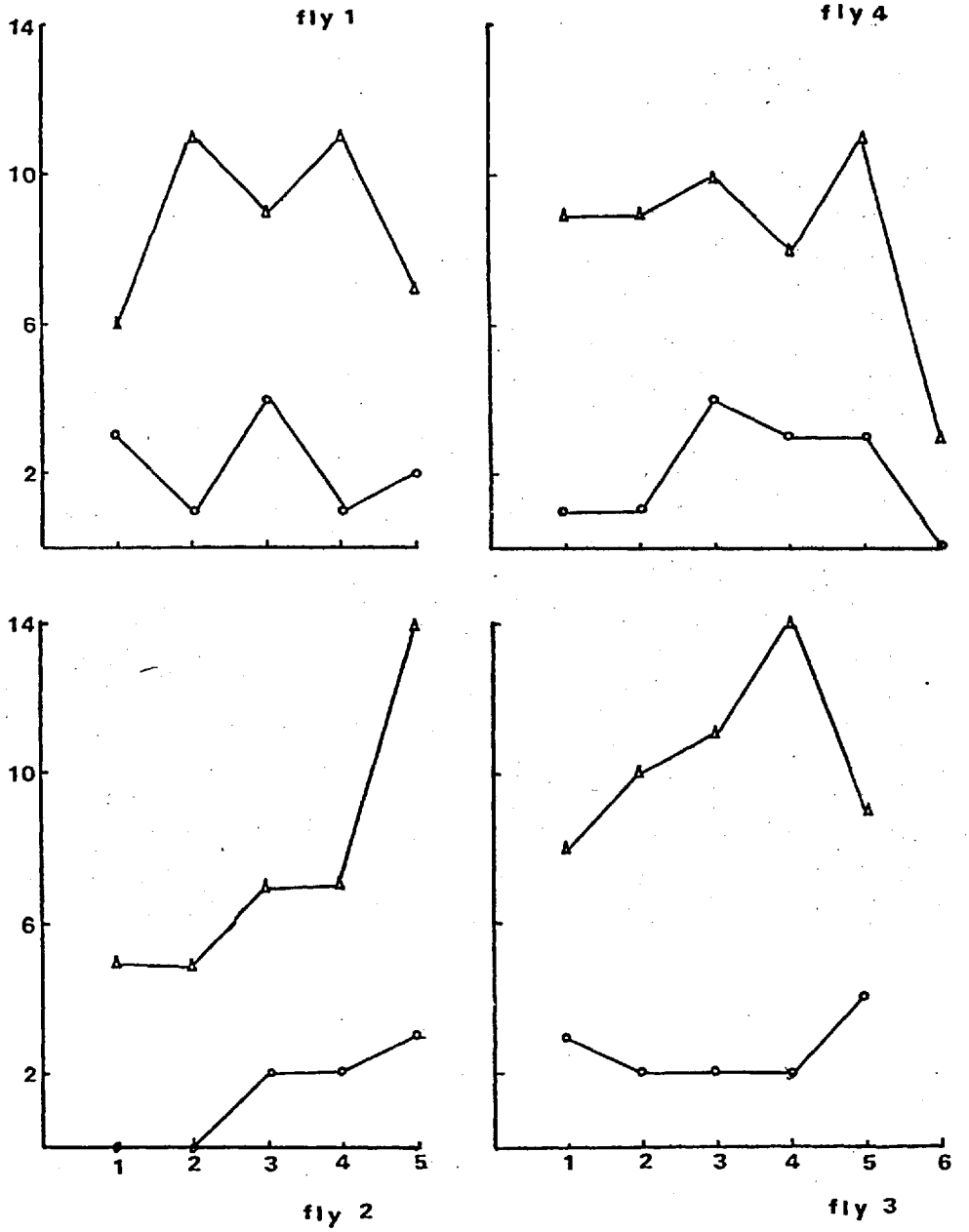
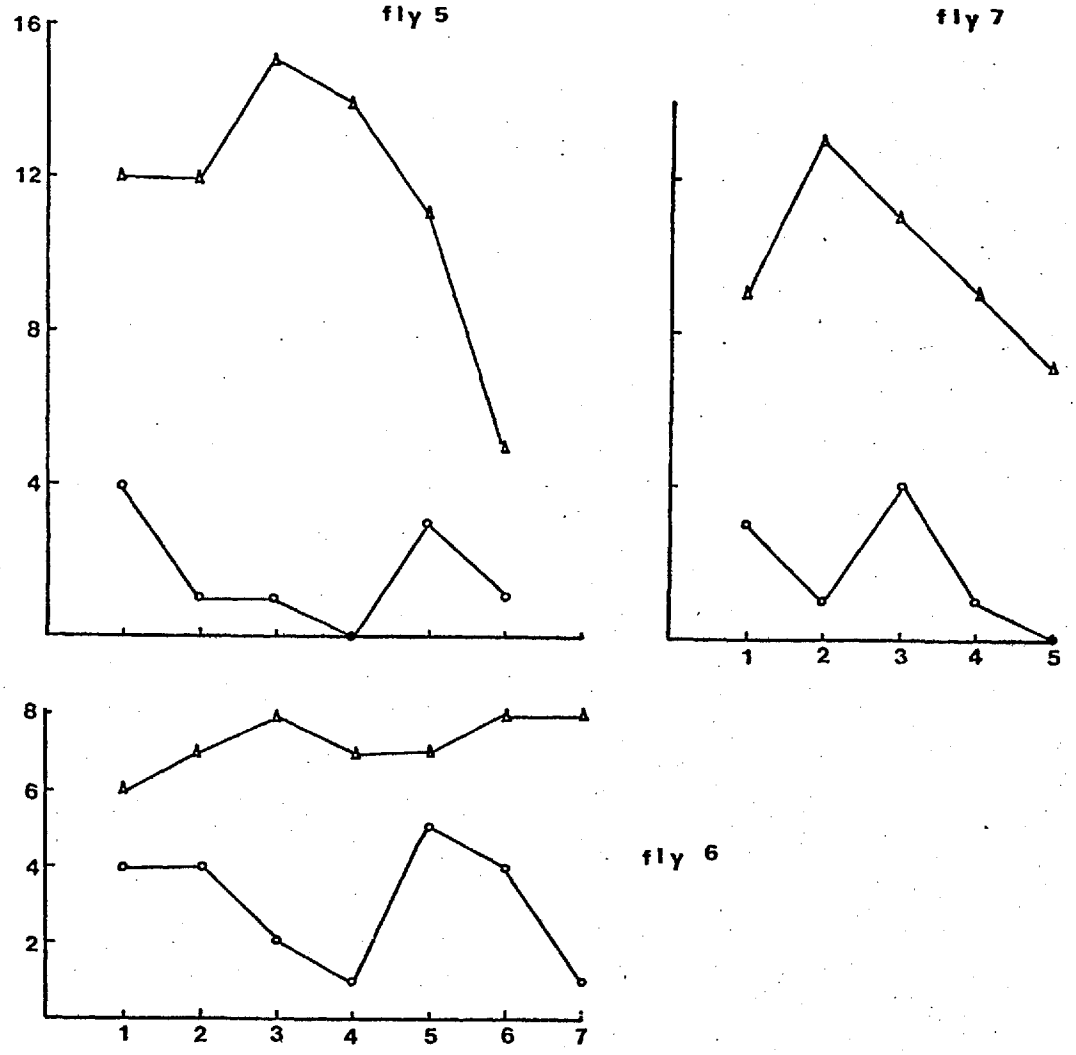


FIG. 9.7. Cont^{d}.



increase in performance until arousal was too high to favour one burst type. Whilst there is general increase in numbers of both burst types across the initial stages of starvation, the increase in performance of one burst at the apparent expense of the other indicates that competition is also an influencing factor.

However, this apparent tendency for bursts of one type to influence the other in some flies is complicated by a further factor. In all flies there is at least one pair of days between which both the number of cleaning bursts and the number of activity bursts increase, e.g. fly 3, days 4 and 5, fly 6 days 1 and 4, fly 2 all days. Thus whilst for these days the percentage increase of one may not match that of the other, they are nonetheless increasing together. The number of joint increase points outweighs the impact of the days of opposite trends, so that a regression of data from all flies between the percentage of the total number of activity or cleaning bursts to occur each day, gave $r^2 = 0.11$, $b = 0.76$, with $p < 0.05$, i.e. a positive association. The data are examined for any influence of performance of one burst type on performance of the other in later sections (9.5.9. and 9.5.10.).

The length of activity bursts and cleaning bursts is shown in Fig.9.6. The length of activity bursts do not remain exactly the same, but vary from day to day. Some of the variation is due to differences in the amount of pre-burst and post-burst cleaning (shown as the discrepancy between truncated and complete burst lengths, and in Fig.9.8.), rather than a difference in activity burst length. However, the truncated lengths do vary and in some, significantly, e.g. t tests on the increase in truncated lengths between days 1 and 5 fly 6 and days 1 and 4 fly 7 gave $P < 0.01$.

FIG. 9.8. MEAN LENGTHS OF PRE- AND POST-BURST CLEANS AND INTER-BURST GAPS

Ordinates: seconds Abscissae: days

Δ, gaps. o, cleans

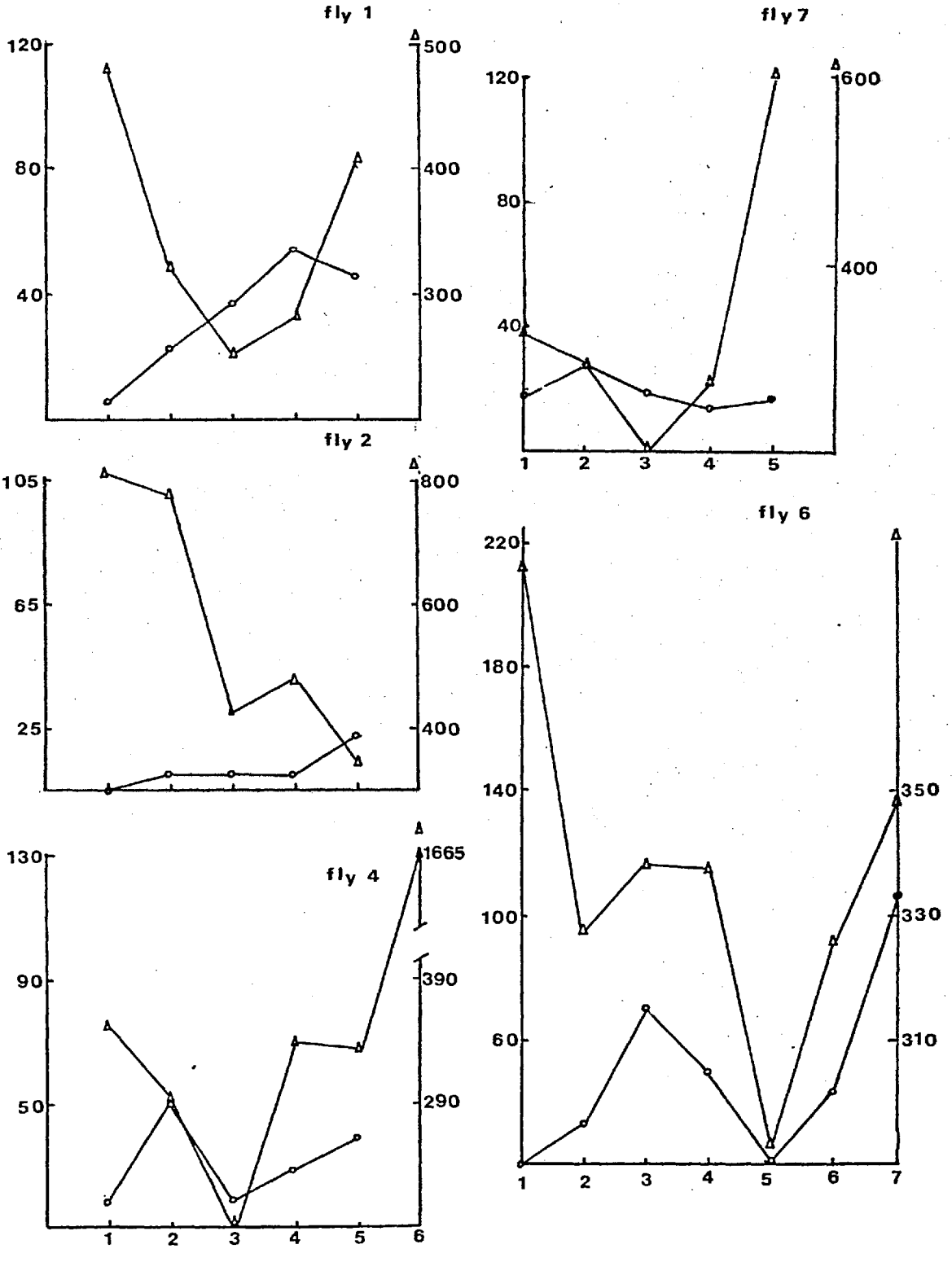
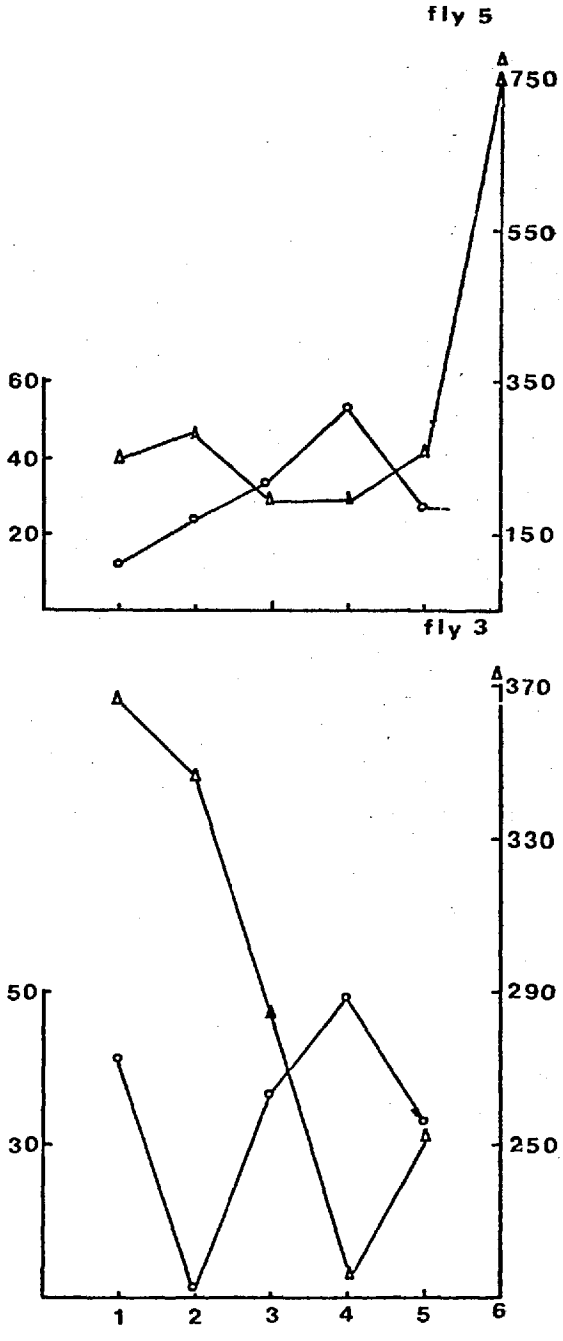


FIG.9.8.Contd.



In flies 1, 3, 5, 6 and 7 there appears to be a trend to increase truncated activity burst length across starvation with a peak being reached after numbers of activity bursts have begun to decline in flies 3, 5 and 7. In flies 2 and 4 there is a steady decline in truncated activity burst length after day 2 whereas in both these flies the number of activity bursts increases until at least day 5. Thus there appear to be two different strategies in response to increasing starvation. In one (flies 2 and 4) part of the increase in numbers of activity bursts is offset by a decrease in length across starvation. In the other,, increased burst lengths augment the increase in burst number and offset some of the activity lost when the numbers decrease until the burst length also declines. This was particularly unexpected since it would imply that proline is still plentiful enough to allow a further increase in flight burst length, so that proline shortage is less likely to be a causal agent for the reduction in activity burst numbers.

The mean length of cleaning burst in all flies except fly 7 shows an increase from the initial short length to peak in the middle of starvation and then decline. A comparison of Figs. 9.6. and 9.7. shows that with the exception of flies 7 and 4, the length of the cleaning bursts is inversely proportional to the number of cleaning bursts.

In a test of this relationship, using data from all flies, the number of cleaning bursts as a percentage of all bursts that day (to compensate for variation in the number of bursts performed by each fly) was used. For the length, the length of each burst as a proportion of the mean length of cleaning burst for each fly (to compensate for variation in the length of burst performed by each fly) was used. The regression between these gave $r^2 = 0.08$, $b = -0.24$, with $\underline{t} = 2.61$ and $p < 0.02$.

There is thus a significant negative regression between length and number of cleaning bursts. Such a reduction of burst length with an increase in arousal has been shown many times, e.g. Hinde (1958b) showed that the closer domesticated canaries were to egg laying, the more complete sequences they performed, and the shorter bout lengths they displayed.

The combined influences of change in burst number and length result in all flies (except number 4) reaching a peak of total performance of locomotor activity on day 4. By contrast, maximum performance of clean occurred on day 1 in 1 fly, day 2 in 2, day 3 in 3 and day 4 in 1, i.e. predominantly earlier. This is shown in Fig.9.9.b.

9.5.5. Examination of lengths of bouts of different behaviour, and of the transitions between bouts.

Within bursts mean bout lengths of individual behaviours follow different trends as shown in Fig.9.5. The mean length of flight bouts is relatively constant, except for fly 4 which showed a steady decrease from 3 to 1 second length after the peak in numbers of activity bursts on day 2. Rest bouts within bursts also remain relatively constant. The length of walking bouts in flies 1, 2, 5 and 6 shows some increase in length to peak on the last day before death. Flies 3, 4 and 7 remain more constant. Cleaning bouts within bursts were highly variable, with fly 1 showing an opposite trend to the steady decrease in length of flies 2 and 4, whilst flies 3, 5, 6 and 7 showed a decrease followed by an increase, and in flies 5 and 6 a further fluctuation.

Within activity bursts the greatest number of transitions were from flight to rest to flight. Within-burst cleaning bouts were also always preceded and followed by rest. However, bouts of walk showed a strong tendency to precede flight rather than to follow it.

The data from all flies combined gave 197 walk bouts, 163 immediately followed by a flight bout, and 34 immediately preceded by one. This last type of walk bout was followed by rest.

9.5.6. Performance of individual bout types - distribution across starvation, and proportion of daily activity occupied by each type

Fig.9.9.a shows the percentage of the total time active each day occupied by each act and Fig.9.9.b shows the percentage of each acts total performance across starvation which was performed on each day. Fig.9.9.a shows that the majority of time spent active was occupied by flight and cleaning. The proportions occupied are mirror images of each other, influenced only a little by the level of walking.

Walking, in spite of an increase in the performance of flight and cleaning, increases the proportion of time active it occupies across starvation in flies 6, 3, 5 and 7, but especially after the performance of clean and flight have begun to decline. This increase in walking late in starvation may reflect a 'frustration' of the tendency to increase flight due to a shortage of proline. Alternatively, walking may be associated with higher arousal levels than flight and thus occur at a later stage in starvation. This is feasible because walking is the last act after alighting on a host and before the stimulus of the blood meal.

Whilst in a system predominated by 2 active behaviours, the proportions occupied by flight and clean were bound to mirror each other, the extent of the divergences, e.g. in flies 2 and 6, indicated that the proportions were not oscillating around some steady balance between the two, but that a large increase in the performance of one was detrimental to the performance of the other (as indicated for numbers of bursts in 9.5.4.). Fig.9.9.b indicates

FIG. 9.9. a) THE PERCENTAGE OF THE TIME ACTIVE EACH DAY OCCUPIED BY THE DIFFERENT BEHAVIOURS, AND TIME ACTIVE AS PERCENTAGE OF THE RECORDING TIME, FOR EACH FLY. b) DAILY PERFORMANCE OF EACH BEHAVIOUR AS A PERCENTAGE OF ITS PERORMANCE ON ALL DAYS FOR EACH FLY

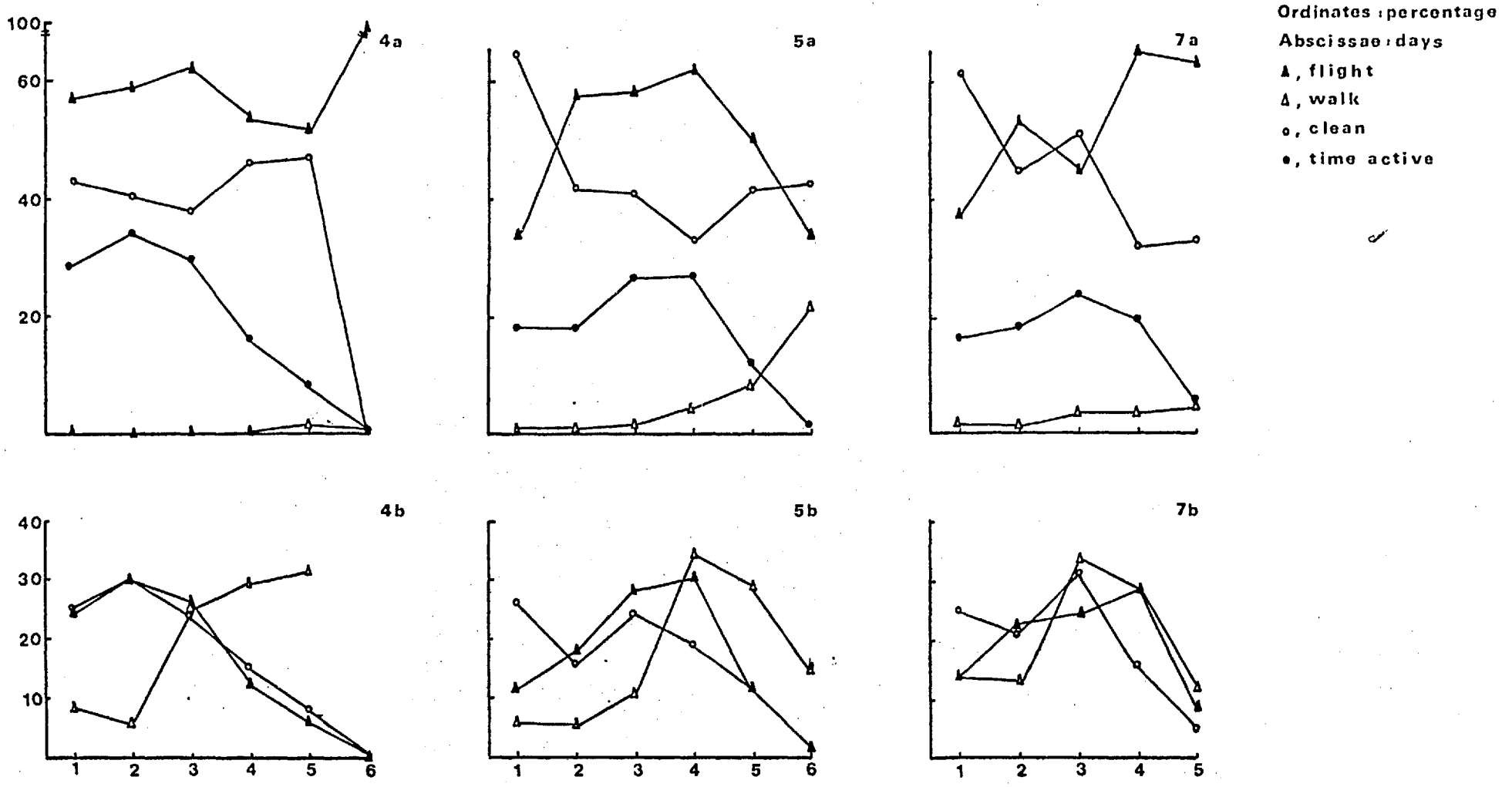
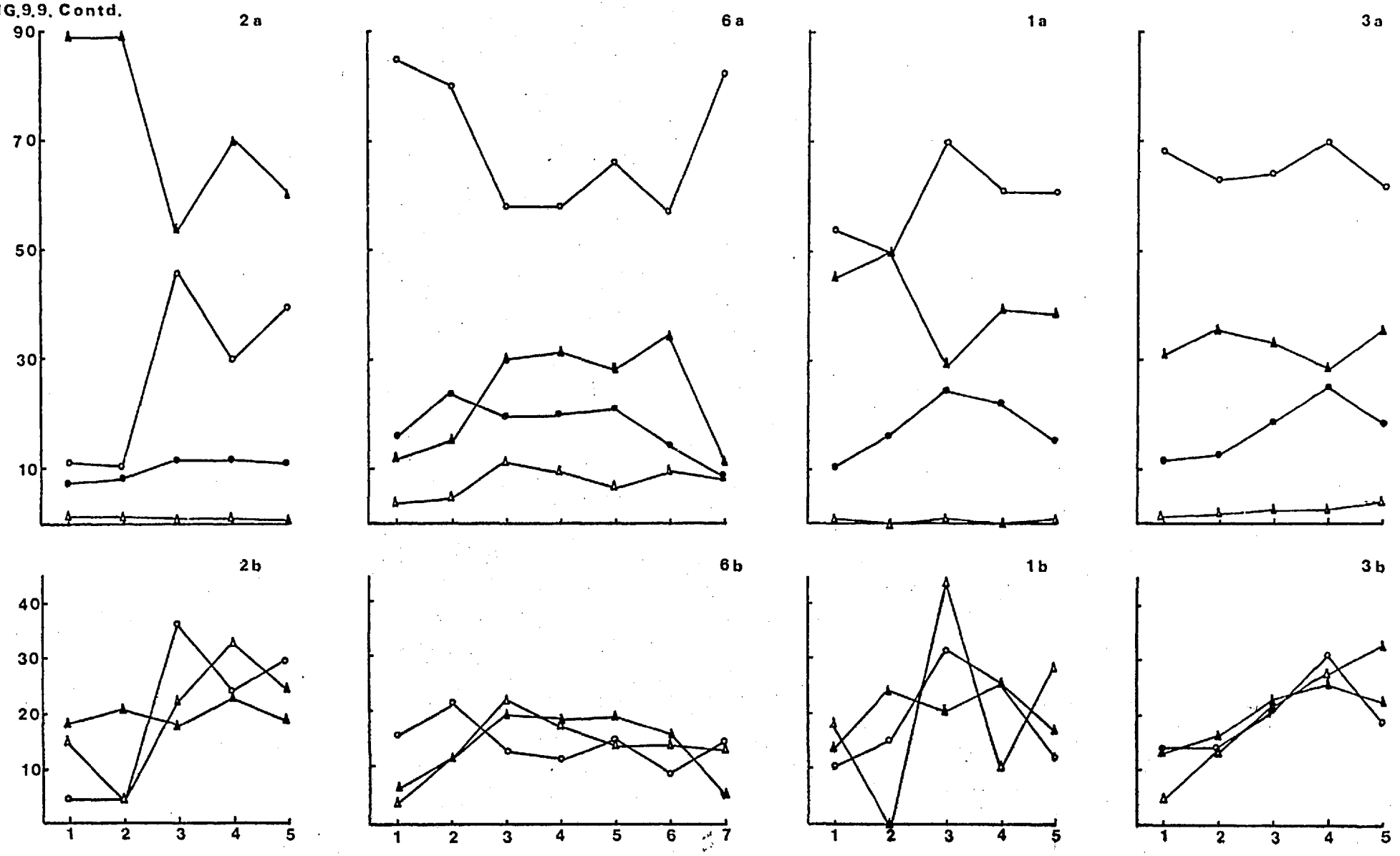


FIG.9.9. Contd.



again that (for flies 1, 2, 5, 6 and 7), performance of flight or clean lowers the probability of performance of the other act for at least one day across starvation.

9.5.7. Summary of results from sections 9.5.1 - 9.5.6.

(1) Log survivor functions of gap length showed that the transition from intra-burst rest bouts to inter-burst gaps occurred at ca. 10 seconds.
(2) The length of cleaning bursts is inversely proportional to the number of cleaning bursts.
(3) Cleaning occurred in 3 different contexts.
(4) There appears to be competition between activity and cleaning bursts for 'performance time' at some times across starvation.
(5) Activity burst mean length was twice that shown in two sets of data from actograph recordings.
(6) Walking bouts increased in mean length, and walking increased the proportion of total time active it occupied, with starvation.
(7) Transitions walk to flight bouts were much more common than flight to walk bouts.
(8) The total performance of cleaning reached a peak in most flies before the total performance of flight.

9.5.8. Further analysis, and qualification of bursts for inclusion

The data from all flies were combined and the results analysed to show if cleaning and activity bursts had an equal effect on the probability of a new activity burst beginning and continuing. Possible relationships between length and proximity of preceding and following activity bursts and the length and time of occurrence of the burst type under investigation were examined.

To make a valid comparison between effects of cleaning and activity bursts, the following procedure was followed. Activity bursts with no pre- and post-burst clean of length greater than 10 seconds (bouts of clean up to this length could be accepted as intra-burst cleaning bouts, see Fig.9.5.) qualified as preceding, middle, and following activity bursts. Individual cleaning bursts were accepted if their preceding and following activity bursts qualified as above. Thus a number of series of activity/activity/activity bursts and activity/cleaning/activity bursts were obtained, where the overall effect of the middle bursts used would be either that of a cleaning burst or of a burst of locomotor activity, with any compound activity burst types excluded. Data from the 7 flies yielded 48 cleaning bursts and 70 flight bursts which fulfilled these requirements. The sequences are illustrated in Fig.9.10.

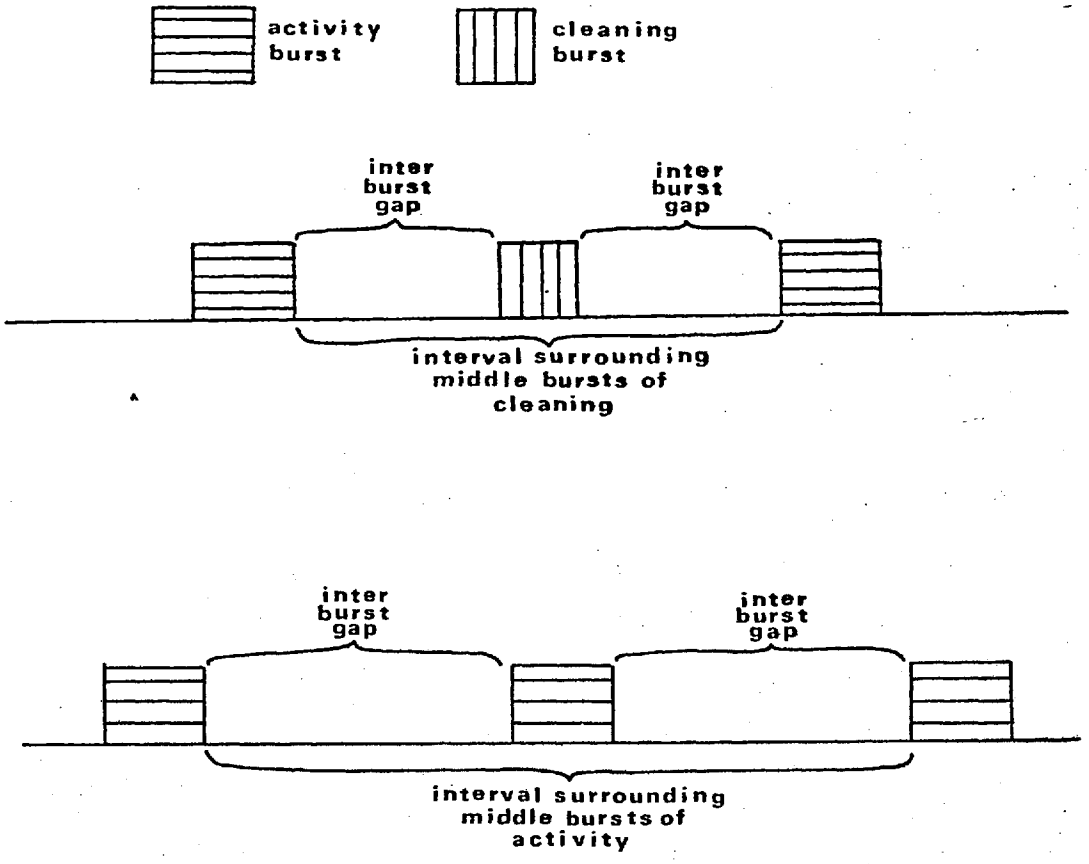
9.5.9. Comparison of the lengths of preceding and following bursts, and intervals, for each middle burst type

The length of all activity bursts preceding cleaning or activity bursts were compared. Since there were more than 30 examples of each burst type, the method described by Bailey (1959) for large samples is used. The results are summarised in Table 9.1.

Table 9.1. Mean lengths (seconds) of activity bursts preceding or following the two middle burst types

	Activity burst preceding	Activity burst following
Cleaning burst	127	127
Activity burst	116	127

FIG.9.10. SEQUENCES OF TRIOS TO ILLUSTRATE THE POSITION OF THE BURSTS AND INTERVAL



The difference between the means of 127 seconds before a cleaning burst, and 116 seconds before an activity burst, was 0.9 standard deviations from zero, with an associated $p > 0.36$. The mean length of activity bursts following cleaning bursts was 126.6 seconds, and following activity bursts was 126.5 seconds. There is thus no significant difference between lengths of activity bursts preceding or following bursts of activity or cleaning.

The time from the end of the preceding activity burst to the beginning of the following activity burst, including the length of the middle burst is hereafter called the interval, (see Fig.9.10.). A comparison was made of the length of such an interval surrounding each middle burst type. The difference between the mean length of intervals around cleaning of 642.8 seconds and the mean of intervals around activity bursts of 924.1 seconds was more than 3.50 standard deviations from zero, with a $p < 0.001$. There is thus a strongly significant difference between the lengths of interval around each type of burst.

Since there is no significant difference between the lengths of activity burst preceding and following clean or activity bursts, the difference in interval length can be partly attributed to the different effects of clean and activity bursts. Since cleaning bursts have shorter intervals, it can be assumed that they have not raised the threshold to locomotor activity so far as an activity burst would have done. However, had the cleaning burst had no effect at all on the threshold, an interval similar to that of a simple activity/activity burst series could be expected, i.e. about half the activity/activity/activity series, ca. 460 seconds. The 640 second cleaning interval exceeds this by ca. 40%. Cleaning therefore exerts some influence on the locomotor threshold, but less than an activity burst itself.

9.5.10. Division of middle bursts into ranks according to the position of their occurrence in the interval

In order to assess further any interaction between different types of burst, it was noted how near in time each type of middle burst occurred to its preceding and following activity bursts. The mean gap length between bursts changes with starvation, and varies between flies (see Fig.9.8. above,), so that use of the actual times involved would necessitate separate treatment of each day to each fly, since each fly did not contribute equal numbers of each type of burst to each rank on each day. To compensate for this, the length of the interval (as defined above) was calculated for each middle burst. The interval was then divided into five equal portions, and each portion assigned a number (from 1 to 5). Each middle burst was thus placed in its interval according to which portion its centre occurred in. The number of the portion was then used as a rank to measure the proximity to the preceding and following bursts. (The centre of the middle burst was used to yield the rank to reduce any bias resulting from the different lengths of bursts, since longer bursts would have spanned a greater number of consecutive time ranks).

The distribution of interval lengths for cleaning and activity bursts of different rank is shown in Table 9.2. For cleaning, there is a consistent increase in the mean interval length from the early

Table 9.2. Mean interval lengths for each burst type ± S.E. (For nos. see Table 9.3.)

Burst type	Rank				
	1	2	3	4	5
Clean	541 ± 50	625 ±124	616 ±101	698 ± 67	752 ±78
Activity	926 ±166	842 ± 91	1055 ±152	944 ±126	274 ±25

bursts to those which occurred later. (a test between ranks 1 and 5 gave $\underline{t} = 2.35$ at df = 18, $p < 0.05$,). The effect of this increase will be to exaggerate slightly the closeness in real time of rank 1's to the preceding burst, and in contrast to decrease slightly the closeness in real time of rank 5 bursts to the following burst. Nevertheless, the ranking retains at least an ordinal meaning. Rank 5's would have occurred on average between 601 and 752 seconds of interval, and rank 1's on average between 0 and 108 seconds of interval. The increase in interval length from rank 3 bursts to ranks 4 and 5, could perhaps be explained if there was a minimum length of rest needed after a cleaning burst to lower the threshold to locomotor activity enough for an activity burst to be initiated.

For flight bursts, there is no obvious trend to increase interval length with rank. The length at rank 5 cannot be viewed with confidence due to the small number of records (2), and is ignored in any further calculations.

Table 9.3. shows the number of clean or activity bursts which occurred at each rank. χ^2 for the number of cleans = 1.00, (at 4df, $p > 0.90$). For activity bursts, $\chi^2 = 42.14$, for which $p < 0.001$.

Table 9.3. Observed number of bursts at each rank

Rank	1	2	3	4	5
Clean	11	7	9	8	9
Activity	5	28	26	9	2

There is thus a closely similar number of bursts in each rank for cleans, indicating a constant random probability that cleans would occur in each rank, and that the proximity of an activity burst has no influence on the threshold to initiation of cleaning bursts. Activity bursts were significantly unevenly distributed however, with a high probability that more bursts occur in ranks 2 and 3, than in 1, 4 or 5. This indicates that there is a fixed length of time needed after a middle activity burst for the threshold to locomotion to fall to a level where a new burst can be initiated.

9.5.11. Comparison of length of middle bursts at each rank

Table 9.4. shows the mean length of each burst type in the different ranks.

Table 9.4. Mean length of each burst type per rank ± S.E.

Burst Type	Rank				
	1	2	3	4	5
Clean	46.8 ±13	88.9 ±28	140.6 ±29	151.9 ±28	87.1 ±21
Activity	129.8 ±25	105.9 ±12	111.2 ±11	139.9 ±19	56 ±24

With bursts of cleaning, there is a steady increase in length with increase in distance from the preceding flight burst until rank 4 is reached. Rank 5 length shows a reversal in this trend, and a return to a mean length typical of rank 2. The difference between length of ranks 1 and 3 gave $t = 3.15$ at 18df, $p < 0.01$. Ranks 1 and 2 and 1 and 5 gave $P > 0.1$. Thus there is a tendency for cleaning bursts to be longer when they are relatively further from flight.

Table 9.3. shows that for cleaning bursts there is an equal probability that cleaning would occur in any rank, whilst Table 9.4. shows that the lengths differed with rank number. This would

indicate that the tendency of a cleaning burst to occur at any time, and the tendency for a burst to continue once begun are not under the same controlling mechanism.

That cleans furthest from activity bursts either side were longest, can perhaps be explained in terms of the level of arousal most suitable for cleaning being the level least suitable for locomotor activity, i.e. a short term 'activity cycle' (see 9.5.4.) is in operation. Fentress (1968a and b), showed that in two species of vole there was a level of arousal which was optimum for cleaning. Rowell (1971b), in a study of antennal cleaning in the locust, found that the occurrence of cleaning was associated with a fall in the DCMD (a visual interneurone) responsiveness, and he suggested that there are some responses whose occurrence is promoted by low arousal. The results given here for cleaning in tsetse flies also indicate that the level of arousal at times furthest away from activity bursts is optimum for continuing performance of cleaning.

Table 9.4. showed that for activity bursts there is no obvious change in length of burst with rank number since the difference between any pair of means gave $p > 0.1$. However, Table 9.3. shows that there was a much greater probability that an activity burst would occur at ranks 2 and 3 than 1, 4 or 5. Thus, for locomotor activity also, it appears that the tendency for a burst to occur in any rank, and the tendency to continue once begun, are not under the same controlling mechanism.

9.5.12. Comparison of length of activity bursts preceding and following middle bursts of different rank

The lengths of preceding and following activity bursts were examined for any differences due to the rank, i.e. the proximity of each type of middle burst. The results are shown in Table 9.5.

Table 9.5. Mean length (seconds) of activity bursts preceding or following bursts of cleaning or activity, ± S.E.

Burst type	Activity bursts preceding						Activity bursts following				
	1	2	3	4	5	Interval rank	1	2	3	4	5
Cleaning	127 ±17	142 ±20	149 ±28	111 ±19	109 ±19		112 ±14	125 ±10	142 ±16	128 ±20	131 ±16
Activity	98 ±30	100 ± 9	123 ±16	153 ±17	[140 ±88]		200 ±46	125 ±17	125 ±10	99 ±16	[116 ±10]

For bursts of cleaning, both preceding and following activity bursts were shortest when adjacent to cleans of ranks 1 and 5, and lohgest adjacent to cleans of rank 3. However, the differences between any pair of means gave a $p > 0.1$, so that it appears that the proximity of a cleaning burst had no significant influence on the length of adjacent activity bursts. Indeed, it would be difficult to envisage a mechanism which would render both activity bursts closely preceded by rank 5 cleans and those followed at a great distance by rank 5 cleans, shorter than those preceded or followed by rank 3 cleans.

By contrast, with middle activity bursts, the mean length of the preceding burst was greater when the middle burst occurred furthest away, i.e. at rank 4, and shortest when the middle burst occurred closely at rank 1. A $\underline{t}$ test between the means at ranks 2 and 4 gave $\underline{t} = 2.75$, at 35 df with $p < 0.01$. Other pairs gave $p > 0.1$. For following activity bursts, again, bursts furthest away from middle activity bursts (at rank 1) were longest at 200 seconds, and those nearest a middle burst (at rank 4) were shortest at 99 seconds. The difference between means after ranks 1 and 4 gave $\underline{t} = 2.56$ at 12 df with $p < 0.05$. The difference between means 1 and 2 gave $\underline{t} = 1.73$, at 31df and $p > 0.05$. Thus it appears that whilst the length of middle activity bursts was not influenced by the proximity of surrounding bursts, (see table 9.4. above), the length of following and preceding bursts of activity decreased with an increase in proximity of the middle burst.

However, clearly activity bursts which were the middle burst of one trio become the preceding ones in the next (unless there is an intervening cleaning burst), so that the different findings are an anomaly. Perhaps it should be noted however, that the results from preceding and following bursts involve twice the number of entries to the middle burst samples, and so perhaps these should be viewed with greater confidence.

To examine the apparent relationship between length of preceding and following bursts and rank of middle burst further, linear regression analysis was used on both preceding and following burst lengths, with both rank (as percentage of interval to pass before the centre of the middle burst was reached), and length in seconds before the centre was reached, this gave a $p > 0.3$ in all cases. For following activity bursts a partial regression between length of following burst and proximity of preceding to eliminate the influence of the length of the preceding still gave $P > 0.1$. Thus there is no simple relationship between lengths of consecutive activity bursts. However the significant difference between lengths of activity burst following middle activity bursts occurring at the two most different ranks, i.e. 1 and 4, indicates that in a sequence of three activity bursts in a row, there is an accumulated rise in the threshold to locomotor activity, resulting in shorter bursts. However, in addition, the small number of activity bursts at ranks 1 and 4 indicates that there is a reduced probability of a burst occurring at all during the period of higher threshold.

9.5.13. Summary of results from sections 9.5.8. - 9.5.12.

(1) Middle cleaning and activity bursts have highly significantly different interval lengths. Cleaning does not have as much effect as locomotor activity on the threshold to locomotion. However, from the preponderence of activity bursts at ranks 2 and 3, there appears to be a fixed period necessary after an activity burst before a further activity burst can be initiated. The proximity of adjacent activity bursts appears to have no influence on the length of middle activity bursts.

(2) It is indicated that there is a minimum length of time needed after a clean for an activity burst to be initiated.

(3) The proximity of activity bursts has no influence on the time of occurrence of cleaning.

(4) There appears to be a level of arousal optimum for the continuation of cleaning, which is furthest away from the level of arousal at which locomotor activity occurs.

(5) The length of adjacent activity bursts is unaffected by the proximity of cleaning bursts.

(6) In a sequence of three activity bursts, the length of the third appears to be influenced by the proximity of the second.

9.6. Results and discussion of Observations of Behaviour in the arena, and comparison with findings from the actograph

9.6.1. Comparison of burst numbers and format

The data from flies in the arena were analysed into bursts as described for flies in the actograph. Fig.9.11. shows the results for seven flies of numbers of bursts of cleaning and activity, and the total performance in seconds of each act, each day.

Activity bursts show a steady increase in numbers from an initial low level, to peak between days 5 and 7. This is very similar to the increase in activity bursts shown by flies in the actograph, except that these reached a peak earlier, between days 3 and 5. The numbers of cleaning bursts were highly variable, and except for fly A did not show a steady increase across starvation. With the exception of fly E, which showed a slight decrease in the total performance of flight across starvation, all flies showed an increase in the total performance of flight per day across starvation. In spite of the lack of a simple trend in numbers of cleaning bursts, the total length of cleaning performanced increased for at least the first four days of starvation in all flies except flies E and G. These trends resemble those shown by flies in the actograph, but the bursts themselves and the relationships between the two burst types, were different in the two situations.

FIG. 9.11. CHANGE IN ACTIVITY LEVEL ACROSS STARVATION OF FLIES IN THE ARENA

Numbers of bursts: ○, cleaning △, activity

Total performance each day: ●, cleaning ▲, flight

Ordinates: left hand, numbers - right hand, seconds

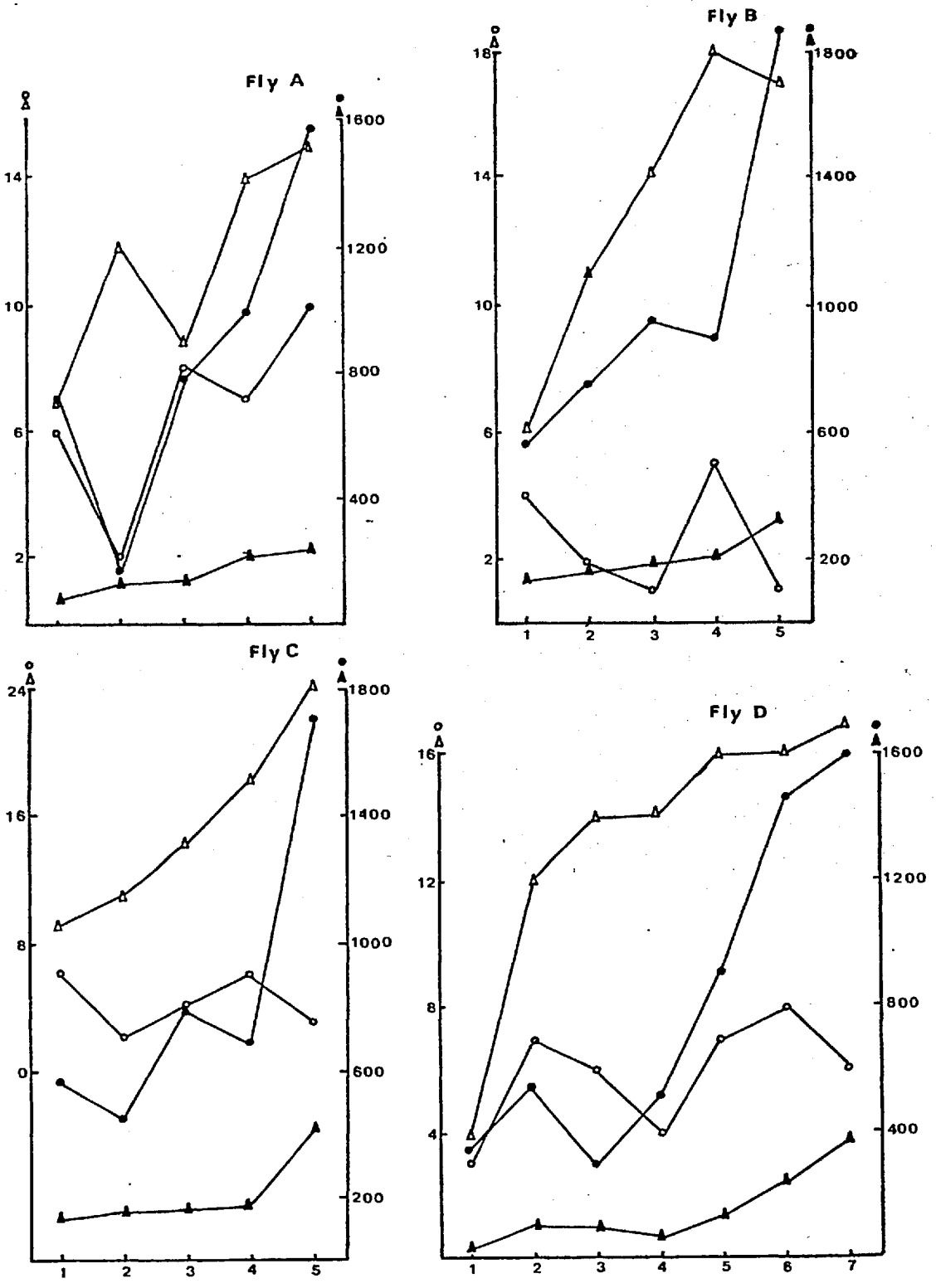


FIG. 9.11. Contd.

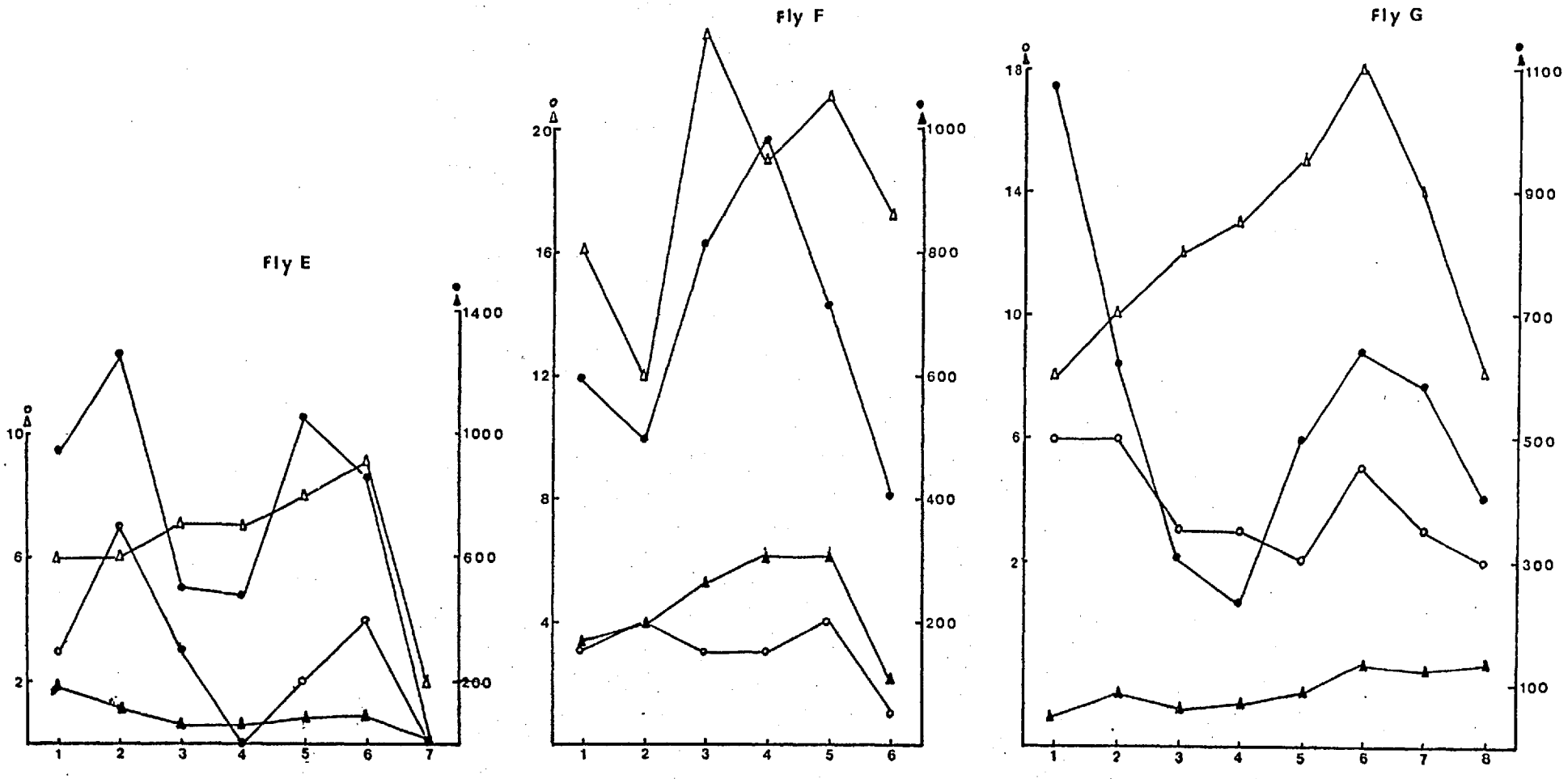


Table 9.6. shows that the mean number of bouts per activity burst, (i.e. the sum of the total of numbers of performances of flight, rest, walk and clean in each burst), of $\bar{x} = 60$ in the actograph and $\bar{x} = 11$ in the arena, was significantly different, $p < 0.001$.

Table 9.7. shows that the mean length of the cleaning bursts in the arena and the actograph were not significantly different ($p > 0.05$) with the mean in the actograph of 116 seconds and that in the arena of 84 seconds. The mean length of activity bursts of 104 seconds in the actograph and 31 seconds in the arena were highly significantly different, ($p < 0.001$), however.

Table 9.6. Mean Number of bouts per activity burst ± S.E.

ACTOGRAPH Fly	Mean Number bouts	ARENA Fly	Mean number bouts	t test
1	54.8 ± 5.6	A	8.21 ± 0.64	
2	67.2 ± 10.7	B	8.7/ ± 0.95	
3	51.7 ± 5.0	C	11.9 ± 1.5	t = 16.3
4	68.9 ± 9.3	D	9.1 ± 1.5	df = 12
5	58.2 ± 11.1	E	12.5 ± 0.98	p< 0.001
6	50.9 ± 8.2	F	15.9 ± 1.85	
7	63.9 ± 10.6	G	8.7 ± 1.95	
$\bar{x}$	59.6 ± 3.3	$\bar{x}$	10.7 ± 0.7	

Table 9.7. Mean length of each burst type

Flies in the Actograph	Length in Seconds		Flies in the Arena	Length in Seconds	
	Cleaning Bursts	Flight Bursts		Cleaning Bursts	Flight Bursts
1	151	116	A	60	35
2	72	121	B	115	37
3	83	98	C	74	26
4	149	126	D	77	32
5	108	80	E	53	36
6	131	89	F	105	38
7	119	101	G	105	16
$\bar{x}$	116 ± 11.6	104 ± 6.5		84 ± 9.2	31 ± 3.0

t tests

cleaning bursts

$t = 2.16$

$df = 12$

$p > 0.05$

activity bursts

$t = 10.22$

$df = 12$

$p < 0.001$.

Table 9.8. shows the differences in length between individual bouts in the two situations. All differences are significant. The mean length of intra-burst rest bouts in the actograph was 1.91 seconds and in the arena 2.33 seconds ($p < 0.05$). The mean length of bouts of walking in the actograph was 0.76 seconds and in the arena 1.41 seconds ($p < 0.02$). The mean length of bouts of intra-burst clean in the actograph was 5.8 seconds and in the arena 13.24 seconds, ($p < 0.001$). The mean length of flight in the actograph was 1.69 seconds and in the arena 4.30 seconds ($p < 0.01$).

Table 9.8. Mean length of individual acts in seconds

Flies in Actograph	Flight	Clean	Walk	Rest	Flies in Arena	Flight	Clean	Walk	Rest
1	1.78	3.71	0.56	2.04	A	8.22	16.4	2.4	2.57
2	1.69	3.89	0.81	1.57	B	5.76	16.75	1.68	2.01
3	1.48	5.09	0.52	1.97	C	2.77	13.6	1.51	1.96
4	2.11	8.69	0.58	1.85	D	4.03	12.54	1.14	3.07
5	1.44	4.45	0.93	1.92	E	4.08	14.54	1.14	2.06
6	1.67	6.1	1.25	2.01	F	2.72	8.95	0.95	2.27
7	1.68	8.7	0.69	2.00	G	2.54	10.00	1.05	2.38
$\bar{x}$	1.69	5.80	0.76	1.91	$\bar{x}$	4.30	13.24	1.41	2.33

Tests for differences in means of actograph and arena

	t	df	p <
Flight	3.33	12	0.01
Clean	5.37	12	0.001
Walk	3.00	12	0.02
Rest	2.64	12	0.05

Thus, individual acts in the arena are significantly longer, but there are many fewer of them per burst, resulting in the mean length of activity burst being significantly shorter. Only isolated cleaning bursts were not significantly different.

The overall effect of fewer, longer flight bouts per activity burst in the arena resulted in less flight being performed per burst. Table 9.9. shows these data. The mean 47.01 seconds/burst in the actograph was highly significantly different from 12.57 seconds/burst in the arena, ($p < 0.001$), with less flight being performed in the arena.

Table 9.9. Mean length of flight per activity burst (seconds) ± S.E.

Flies in Actograph		Flies in Arena		t test
1	42.9 ± 0.64	A	16.02 ± 0.98	
2	55.94 ± 9.16	B	15.73 ± 1.79	
3	28.73 ± 2.40	C	11.9 ± 1.65	$t = 5.14$
4	80.47 ± 17.72	D	10.68 ± 2.12	$df = 12$
5	43.96 ± 6.81	E	13.38 ± 3.36	$p < 0.001$.
6	30.32 ± 5.05	F	12.49 ± 1.59	
7	46.73 ± 7.83	G	7.82 ± 1.27	
$\bar{x}$	47.01 ± 6.62	$\bar{x}$	12.57 ± 1.08	

The difference in the lengths of activity bursts in the two situations indicates that there is no tendency to perform spontaneous activity bursts of a standard length, irrespective of the situation. Earlier (see section 6.1.2.), flies in actographs, which could only walk and hop showed bursts of a similar length to activity bursts performed by flies capable of flight, also in actographs, thus indicating a standard burst length. Presumably the greatly enlarged volume of the arena rendered some factor active in influencing burst length, which was not operative in the actograph.

The utilisation of proline, the main flight metabolite, may reasonably be considered as one factor likely to be involved in the termination of an activity burst. If proline was the only factor involved, these data indicate that continuous flight was more 'expensive' than shorter flights where take off and landing occupy a greater proportion of the time occupied by flight, resulting in burst termination after less flight activity.

In addition, these data indicate that if the threshold to flight is involved in burst termination, then this threshold is raised more by a given number of seconds of flight performed continuously than by the same number of seconds performed at a number of different times.

The arena offered a much greater area for flight before an obstacle such as a wall was encountered, and the unsuitable nature of the actograph for flight may further explain the observed differences. The greater number of transitions per unit time in the actograph from bouts of one act to bouts of another may indicate a greater central excitability, due to the continued frustration of the flight tendency. Such 'post-inhibitory rebound' has been recognised and recorded (Kennedy, e.g. 1965). However, for the performance of spontaneous flight, there are no obvious stimuli, measurement of which could be used to quantify this further.

9.6.2. Comparison of relative performance of each act

Table 9.10 shows that there are significant differences between the percentage of the total time active occupied by each act, except for walking, which in the actograph occupied a mean 3.1% and in the arena 1.44%, with $p > 0.1$. The mean of 50% for cleaning in the actograph and 82% in the arena gave $p < .0.001$, and correspondingly, the mean for flight in the actograph of 47% and 16% in the arena gave a $p < 0.001$.

Table 9.10. Overall % of total time active occupied by each act

Flies in Actograph	Clean	Flight	Walk	Flies in Arena	Clean	Flight	Walk
1	59.2	40.2	1.0	A	81.8	16.2	1.9
2	27.3	72.0	0.8	B	74.8	23.8	1.4
3	65.4	32.4	2.31	C	81.0	18.0	0.1
4	43.0	56.5	0.44	D	83.3	15.3	1.4
5	44.4	49.3	6.32	E	88.6	10.5	0.9
6	69.4	23.01	7.56	F	71.7	24.0	4.3.
7	44.4	52.4	3.20	G	95.9	4.0	0.1
$\bar{x}$	50.4	46.5	3.1	$\bar{x}$	82.4	16.0	1.4

Tests for differences in means in actograph and arena

	t	df	p
Clean	5.0	12	< 0.001
Flight	4.56	12	< 0.001
Walk	1.38	12	> 0.1

Cleaning therefore occupied a significantly greater proportion of the total time active in the arena than in the actograph. This was unexpected, since the confined space of the actograph resulting in more frequent contact with the netting, could have been expected to increase the amount of cleaning as a result of the extra irritation. The lengths of isolated cleans in the actograph and arena however, are not significantly different, so that the burst structure of these isolated cleans was unaffected by the environment.

9.6.3. Summary

Thus in the arena activity is performed in bursts interspersed with long gaps with no visible activity, similar to the arrangement in actographs. Whilst the cleaning bursts are similar from the two situations, activity burst format was highly significantly different. Proline level, flight threshold and the unsuitability of actographs for continued flight are factors likely to be involved in determining the observed difference. These data alone do not allow any further clarification of relative importance of the different factors.

9.7. Results and discussion of spontaneous activity bursts alternated with periods of visual stimulation

9.7.1. Measures used to indicate any change in the tendency to perform spontaneous or stimulated activity.

In order to establish whether the tendency to perform bursts of spontaneous locomotor activity, and the tendency to respond to visual stimulation changed in parallel across starvation, a measure of each tendency was calculated.

Previous analysis of observations of activity in the actograph had shown that activity burst length did not change across starvation in a similar manner in different flies (see Fig.9.6.). However, the gap between bursts (see Fig.9.8.) showed a steady decrease in length with an increase in number of bursts. Brady (1972a, 1975) had also shown that flies whose activity was measured by recording the rocking of actographs, achieved an increase in activity level by a modulation of their periods of inactivity. Measurement of any change in length of such a gap should therefore give a good measure of the prevailing tendency to perform spontaneous activity bursts.

Under the method used, two inter-burst gap lengths were measured. The first was between the end of the first activity burst and onset of the second, both bursts occurring spontaneously. The second spontaneous activity burst was closely followed by a period of visual stimulation. However, the second gap type, from the end of visual stimulation to the onset of the third spontaneously performed activity burst, could not be used. Variation in response to visual stimulation meant that this second gap type followed periods of inactivity during stimulation varying from 0-93 seconds. In view of this, only the first gap was used as a measure of the interval between two bursts of spontaneous locomotor activity.

Analysis of activity during visual stimulation yielded three associated measurements. There was usually some latency of response between the appearance of the stripe, and initiation of the flight response. For flies which showed no response at all, this 'latency' would last the entire six passages of the black stripe lasting 93 seconds. Excluding any flies which gave no response at all in 93 seconds, the mean of 41 recordings of the latency period for all flies all days for the first period of visual stimulation (immediately after the second spontaneous activity burst) was 9.34 ± 2.78 seconds, with only 5 out of the 41 being greater than 10 seconds in length. For the second period of visual stimulation (mid-way between two bursts of spontaneous activity), the mean of 30 recordings was 6.91 ± 2.01, with only 2 out of the 30 being greater than 10 seconds in length. (The smaller number of available records is a result of the greater number of non-response times of 93 seconds).

There was often a gap between cessation of flight response, and the final disappearance of the stripe. Such habituation times varied with starvation, so that early on habituation occurred quickly, with flies responding to only one or two traverses of the stripe. This would yield post-habituation times of 30, 40 or 50 seconds in length (i.e. a great proportion of the 93 second total recording time). Late in starvation flight activity would often continue

until the final disappearance of the stripe, and occasionally for a short period after this. From 112 observations there were 28 such occasions, which gave a mean length of flight of 4.7 ± 0.9 seconds.

Thus, initial response to the stripe tended to be an 'all or none' event with very short latency periods, with varying thresholds being reflected in the change in habituation times across starvation. Fig 9.12 shows the change in habituation times across starvation for all flies for the two periods of stimulation. It can be seen from the slope of these lines and the magnitude of times involved that the increase in time to habituate will account for the majority of the change in length of response time shown in Fig.9.13.

During flight response to visual stimulation, a fly would first take off at some point during the time the stripe was visible, and then would often land just as the stripe disappeared. The fly would then take off again either before the stripe reappeared, or just as it did so. The time in between these flights was spent in a posture of 'attentiveness', with the fly looking towards the site of disappearance of the stripe, and with its body held fractionally less close to the substrate than at ordinary rest. Such take offs, flights and landings would continue until habituation occurred.

The time from the first take off in response to the stripe to the final landing which ended the series of flight responses, is thus a measure of responsiveness to the length of visual stimulation offered. The length of response to the visual stimulation offered immediately after the second spontaneous activity burst is hereafter called R1, and the response to the visual stimulation offered between two spontaneous bursts is hereafter called R2.

FIG. 9.12. THE CHANGE WITH STARVATION IN TIME AFTER HABITUATION TO THE VISUAL STIMULUS

Δ, ○, Length of time after habituation R2, R1, respectively.

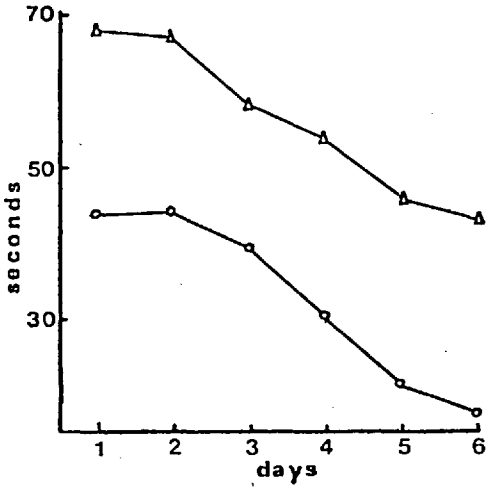


FIG. 9.13a THE CHANGE WITH STARVATION IN THE LENGTH OF THE GAP BETWEEN THE FIRST AND SECOND ACTIVITY BURSTS, AND IN RESPONSIVENESS TO VISUAL STIMULATION IMMEDIATELY AFTER AN ACTIVITY BURST (R1), OR MIDWAY BETWEEN TWO SUCH BURSTS (R2)

Ordinates: seconds Abscissae: days

□, gap length. ○, R1. Δ, R2

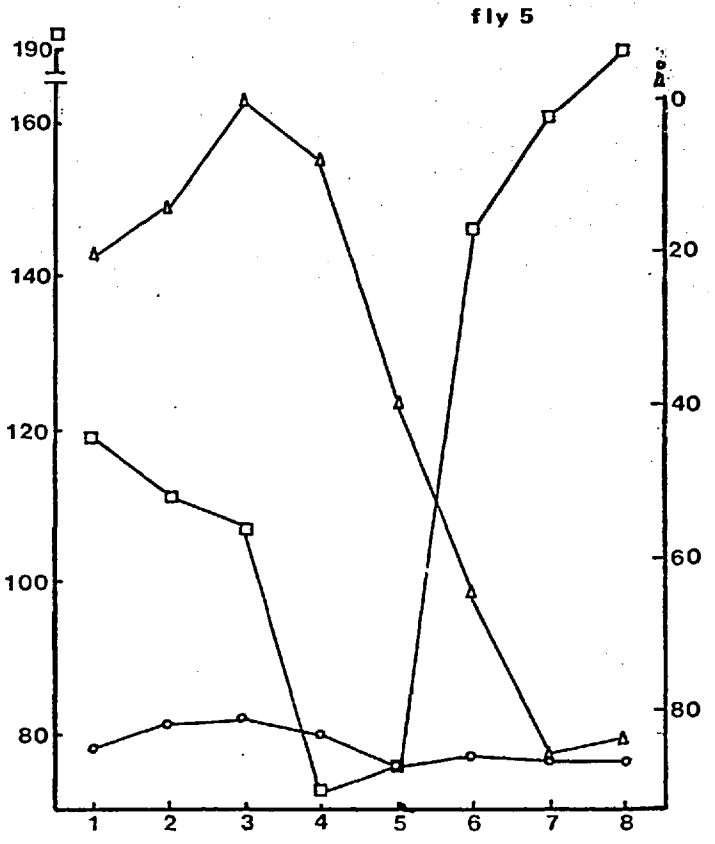


FIG. 9.13a. Contd.

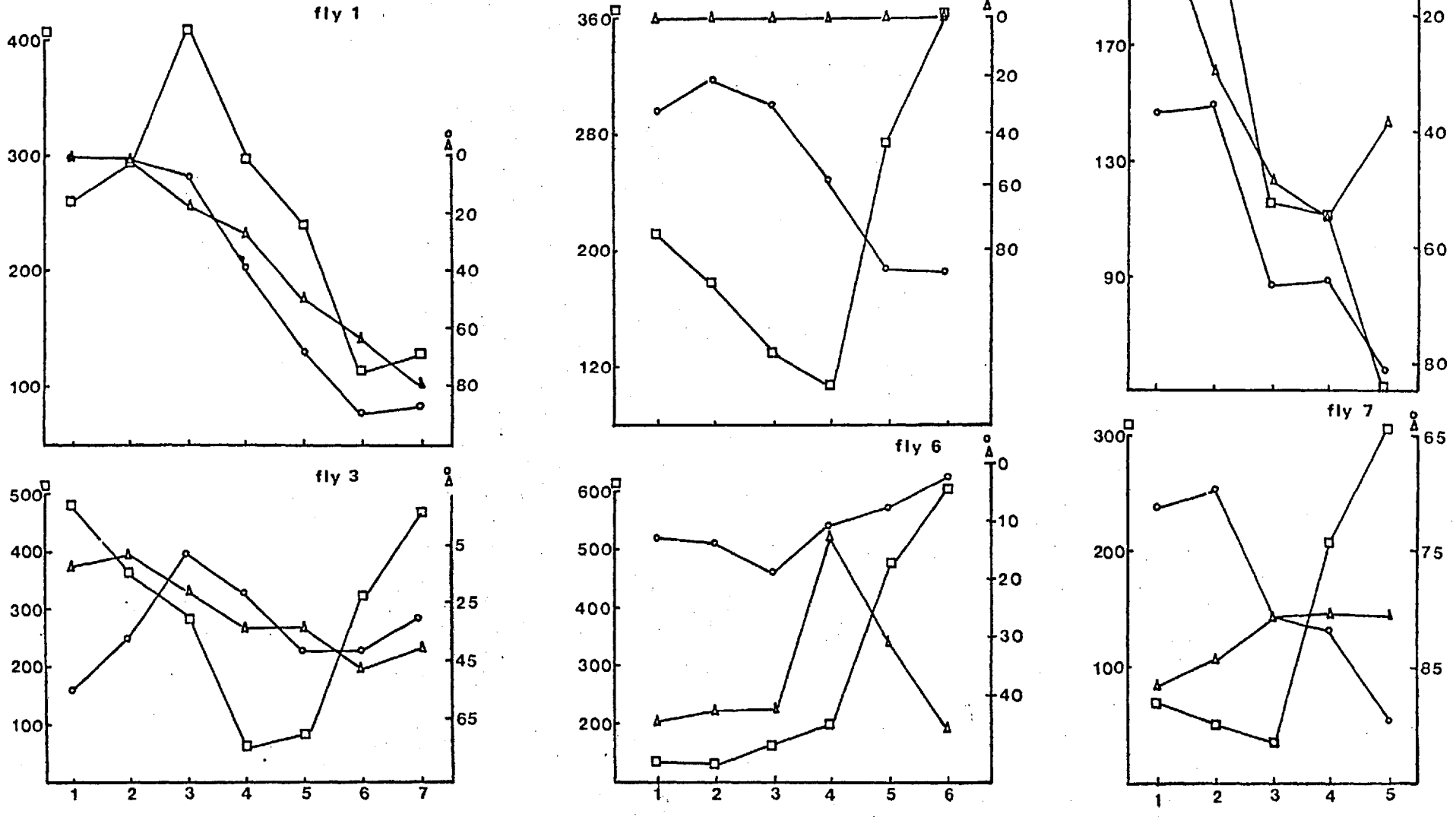


FIG. 9.13 a. Contd.

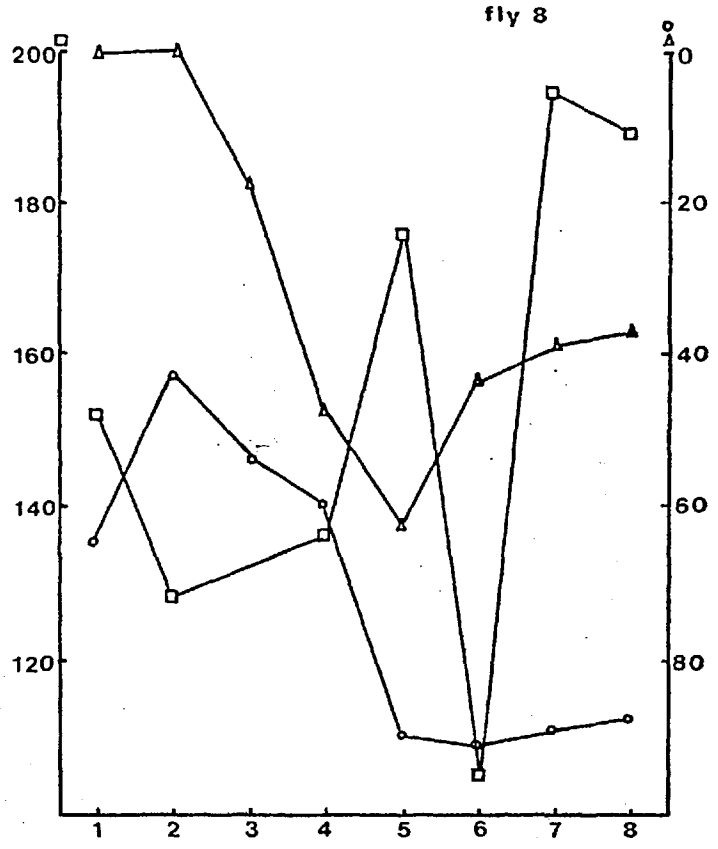
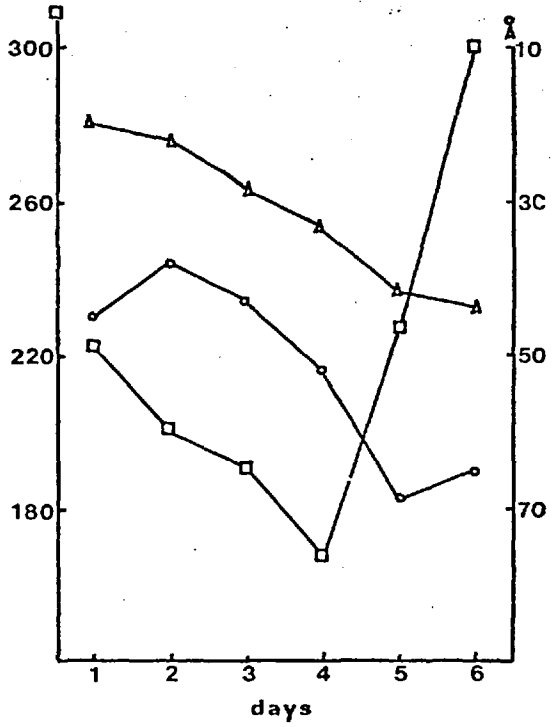


FIG. 9.13b. THE CHANGE WITH STARVATION IN GAP LENGTH, R1 AND R2, MEAN DATA ALL FLIES

Ordinates: seconds. □, gap length. ○, R1. △, R1.



9.7.2. Observed changes in the two measures

Fig.9.13a. shows the change in responsiveness and spontaneous activity across starvation in individual flies, and Fig.9.13b. shows the means of data from all flies. For ease of comparison, the ordinate for time of response is inverted in both parts of Fig.9.13, so that a decrease in gap length with starvation is seen parallel to the increase in lengths of response with starvation. All curves have been smoothed with three point sliding means. With the exception of Fly 6 (which showed a steady increase in the length of the first gap, and a steady decrease in the length of R1), all flies showed a reduction in gap length with starvation, to reach a trough on days 3, 4, 5, or 6, preceding an increase in gap length with further starvation.

Flies 1, 3 and 4 showed a close relationship between the decrease in gap and increase in response lengths across the middle portion of the recordings. In flies 2 and 7, decrease in gap length and increase in R1 followed similar patterns until the gap length began to increase. In flies 5 and 8, the change of R2 followed gap length more closely than did R1. In five of the flies (2, 3, 5, 7 and 8), R1 continued to increase, or remained constant after gap length had begun to increase. In five of the flies (1, 3, 5, 6 and 7), R2 continued to increase, or remained constant after gap length had begun to increase. Five of the flies (1, 2, 4, 5 and 8) show R2 to be consistently shorter than R1.

The mean data from all flies in Fig.9.13b. show a steady decrease in gap length to day 4, and a sharp increase thereafter. R2 is always shorter than R1, with some 20 seconds difference, and both increase steadily across starvation.

9.7.3. Discussion of relative lengths R1 and R2.

The difference indicates that in R1, in spite of the very recent termination of the spontaneous activity burst, the threshold to flight as a response to visual stimulation was lower than at the time mid-way between bursts which gave R2. Fentress (1968a and b), in a study of two species of vole had found that an overhead moving object was more likely to clause fleeing in animals which were, or had recently been, engaged in locomotion. He suggested from these data that the tendency to perform a behaviour may persist for some time after that behaviour is interrupted and replaced by another activity. The results from the flies given here, where post activity burst stimulation could evoke further flight, indicate that termination of a spontaneous activity burst is not caused by an absolute shortage of proline. (If proline level is involved in termination of spontaneous activity bursts then it must be via attainment of a low 'indicator' level of thoracic proline, or by achievement of a required increment decrease from the level at burst initiation to that at burst termination). The greater R1 values indicate that the threshold for flight performance is not raised to that typical of R2 as soon as rest has become the ongoing dominant activity. Thus, for tsetse flies in this situation, the tendency to perform flight continues for some time after cessation of spontaneous flight in an activity burst.

That absolute shortage of proline is not a causal factor in burst termination, and the flight threshold is not raised immediately the burst is terminated would indicate a central overriding control of burst length rather than a peripheral one. Data from Chapter 6 showed teneral flies with punctured ptilina performed walking bursts of similar length to the flight bursts of other tenerals. This is another indication that the length of spontaneous activity bursts are centrally controlled, irrespective of immediate energy expenditure and proline availability.

The low values of R2 indicate a low level of responsiveness mid-way across an inter-burst gap. The log survivor functions shown under the actograph observations indicated a tendency for spontaneous activity bursts to occur at regular intervals, i.e. there was a predictable decrease in flight threshold across the inter-burst gap. That R2 values were low at a period one third of the way through the inter-burst gap (see method) supports the proposal that the flight threshold risss to a peak some time after a spontaneous activity burst, and then gradually decreases until a further burst is initiated.

9.7.4. Discussion of relative changes of spontaneous activity level and visual responsiveness

Apart from a small initial decrease in R1, both R1 and R2 increase in length to day 4 in a trend similar to the decrease in gap length. However, there is no decrease in R1 or R2 to mirror the sharp increase in gap length on days 5 and 6. R2 continued to increase to day 6, whilst R1 continued to increase to day 5, with a tailing off to day 6.

These data indicate that up to day four, visual responsiveness and spontaneous activity level were under the same controlling mechanism (see also Brady, 1975). From day four onwards, it appears that some limiting factor begins to act on spontaneous activity level, but does not affect visual responsiveness.

It has been suggested (see Chapter 7, this thesis) that the reduction in number of spontaneous flight bursts in late stages of starvation may be explained as follows. The time after each flight burst needed for the proline level to return to the minimum level needed for initiation of a new spontaneous burst increases with starvation. This results in a decrease in the number of bursts, in spite of the increased urgency of finding a bloodmeal with increased starvation. The failure of

R1 and R2 to mirror the post day four increase in gap length with a decrease, would indicate that there is effectively no minimum proline requirement for response to visual stimulation. This is supported by the fact that R1, immediately after a spontaneous flight burst which will have depleted proline levels, was always longer than R2 which occurred at a time when the proline reserve would have been partially reconstituted. To a fly in its natural environment, this control mechanism would have the effect of conserving proline from possible wasted depletion via spontaneous activity, whilst not affecting the response to a visual stimulus which would have a higher probability of resulting in a bloodmeal.

In Chapter 8 data are presented which show that probing responsiveness to a suitable temperature also continues to increase until death point. This it would seem that in tsetse flies, responsiveness to relevant exogenous stimuli continues to rise right up until death, whereas response to the (assumed) continuous fall in threshold to spontaneous activity bursts (possibly by the mechanism of minimum proline requirement), is reduced, some days before death from starvation.

Summary

An investigation of physiological parameters which were potentially involved in a causal relationship with known changes in responsiveness of G.morsitans across starvation was made.

Measurement of the osmotic pressure of the haemolymph of mature flies across starvation showed it to be regulated, at least until very late in starvation, and thus not involved in the behavioural changes.

Previous work had shown whole fly weight to correlate with changes in spontaneous locomotor activity. Wing-clipping to give an apparent increase in weight on the wing, did not result in an associated change in activity. Wings are thus unlikely to be involved in monitoring weight. Any change across starvation in the tendency of flies to hold balls of different weights by their legs was measured, and this showed a trend to decrease the weight retained. The decrease was reversible by feeding, and there was a weak correlation between the percentage change and the amount of food imbibed. Tsetse fly legs are thus sensitive to different weights, and it is concluded that they are responsible for monitoring weight changes across starvation.

One common stimulus preceding the similar changes in behaviour with starvation of teneral and mature flies is the expansion of the crop with air at emergence, or with the bloodmeal, respectively. For teneral flies with ptilina punctured at emergence, the behaviour of flies which were able to proceed with crop expansion and wing extension was compared with the behaviour of flies with unextended wings. Flies with extended wings showed less locomotor activity initially and then an increase with starvation, whereas flies with unextended wings performed most activity early. Thus

for teneral flies it appears that crop expansion at emergence sets a high locomotor threshold prior to the observed increase in activity. Flies from both treatments showed bursts of activity of similar length, in spite of the differences in mode of locomotion and energy utilisation, indicating the length of such spontaneous bursts to be centrally controlled.

For mature flies, groups were fed different dilutions of blood through a membrane system, and then the bloodmeal sizes, the rates of diuresis and the change in behaviour with starvation compared. Bloodmeal sizes and thus crop stretch were similar, showing no immediate compensation for dilution. The rate of diuresis also showed no adaptation, with extra liquid being discarded by a continuation of diuresis at a standard rate. The total number of flight bursts performed by each group across starvation reflected the amount of nutrient remaining after a meal. The distribution of flight bursts in the two groups differed, with an initial high threshold to flight and subsequent increase in activity in flies fed the most concentrated blood, and an initial low threshold to flight and subsequent decrease in activity in flies fed more diluted blood. This would be expected if the different weight of nutrient reserve remaining in each group after diuresis influenced the threshold to locomotor activity. The similar input of length of time feeding and crop stretch in each group had no effect - this last finding for the mature flies in contrast to that for tenerals above.

Flies fed either partial meals at two different times after the second bloodmeal, or a full meal, all reached a common base line level of activity immediately after the meals. It is proposed that a common shortage of proline (from metabolic costs proportional to the meal size) accounted for the shared activity level. Where the meal had been large enough to allow a proline reserve to form, there was then an increase in activity with starvation, as would be expected from a threshold set by changing

weight of the fly as that reserve was depleted. Activity of fully fed flies continued to increase after thoracic proline levels would have reached a maximum level, and thus the level of proline is not excitatory. Where the meal had been too small to allow reserves to accumulate, or after such a reserve had been depleted, in spite of weight decrease with starvation, activity showed a decrease, assumed to be due to a shortage of proline. Activity began decreasing from different levels in the three groups, enabling the rates of decline to be very similar.

Detailed recordings were made of some behavioural changes with starvation. The probing response to five different temperatures was measured. Responses to the temperature stimulus from a glass substrate or from a small heated ball were similar. Few flies probed immediately after the third bloodmeal. As starvation increased, a greater number of flies probed, and the range of response (in both the number of flies probing and the length of time taken to probe) to the different temperatures was greatest two days before death. By this time, response to the preferred temperatures of 37°C and 42°C had reached the maximum observed. By the final day before death, the less preferred temperatures of 47°C and 32°C had become more acceptable, responses to them were closer to the maximum, and the range narrowed. Probing responsiveness, unlike spontaneous locomotor activity, increased until the last day of life.

In an investigation of the change with starvation in the spontaneous performance of different acts, recordings were made of observations of individual flies in an actograph and an arena.

In the actograph, activity bursts were performed which consisted of 20-30 bouts of flight alternated with rest bouts, and interspersed with walk and clean bouts. Cleaning occurred in three different contexts, each with a typical length. The

numbers of activity bursts showed a steady increase across starvation, but the number of cleaning bursts was variable. There was a strong negative correlation between the number and length of cleaning bursts. The number of activity and cleaning bursts on any day seemed to be inversely related, perhaps due to competition for performance time or to varying levels of arousal differentially favouring one burst type.

In the arena, activity bursts again showed a steady increase across starvation, but reached a peak two days later than in the actograph. Cleaning bursts were of similar length in the arena and actograph, but there were significantly fewer, longer, bouts per activity burst in the arena, resulting in the bursts being shorter. There was thus no tendency to perform bursts of activity of a standard length in both the arena and the actograph, in contrast to the findings for tenerals with punctured ptilina in actographs. It is assumed that the extra volume of the arena, allowing longer flight bouts, rendered some factor active in influencing burst length which was not operative in the actograph.

Data from the actograph were examined for any influence of the burst types on each other. Measurement of intervals within triplet burst sequences showed that cleaning bursts had some influence on the threshold to locomotor activity, but not as much as an activity burst would have had. For both cleaning and activity bursts, the tendency for a burst to begin and the tendency to continue seemed to be under different controlling mechanisms. The length of cleaning bursts were influenced by the proximity of activity bursts, but activity bursts of standard length were performed, irrespective of the proximity of cleaning bursts.

A comparison was made of changes in the tendency to perform spontaneous locomotor activity and in visual responsiveness across the gap between two activity bursts, and across starvation.

Responsiveness to the visual stimulus was greater immediately after the end of an activity burst than midway through the gap, indicating that the threshold to locomotor activity does not reach a peak until sometime after the end of an activity burst. The ability of flies to respond immediately after an activity burst indicates that such bursts are not terminated due to an absolute shortage of proline. The level of spontaneous locomotor activity reached a peak on day 4 of starvation, and then decreased. Visual responsiveness also increased to day 4, but then continued to increase until death as does probing responsiveness. It is proposed that a minimum level of proline needed for initiation of a spontaneous activity burst but not for visual responsiveness might be a mechanism whereby the observed difference between the two responses is controlled.

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APPENDIX 1. Standarisation of flies for emergence weights and nutritional history

Experiment	Trials			Range of emergence weights in mg	Range of mg blood imbibed all 3 meals/ mg emergence weight	Range of mg blood imbibed 3rd meal/mg emergence weight
Spontaneous locomotor activity	Mature males			16.6-25.1	5.0-6.9	1.8-2.5
	Teneral males			20.7-21.5		
Wing clipping	Males	a	clip	18.6-23.6	4.5-6.6	1.4-2.2
			control	16.4-24.8	5.2-6.4	1.8-2.7
		b	clip	14.9-22.5	5.3-6.4	1.8-2.5
			control	18.1-23.4	5.3-6.3	1.8-2.1
		c	clip	16.6-20.1	5.2-6.2	1.8-2.7
			control	14.0-25.8	5.2-7.9	1.8-2.8
		d	clip	14.9-23.6	4.5-6.6	1.4-2.7
			control	14.0-25.8	5.2-7.9	1.8-2.8
	Females		clip	17.6-25.2	6.0-8.3	2.2-3.0
			control	17.4-24.5	5.6-8.0	2.1-3.0

Experiment	Trials	Range of emergence weights in mg	Range of mg blood imbibed all 3 meals/ mg emergence weight	Range of mg blood imbibed 3rd meal/mg emergence weight
Leg loading	Teneral Males	18.4-24.4		
	Teneral females	19.6-25.4		
	Mature males			
	29 permanently mounted	15.3-23.6	3.9-6.17	0.87-2.67
	20 string mounted	18.3-25.3	4.08-6.83	0.92-2.45
	Mature females	18.5-24.4	4.74-6.17	1.05-2.74
Osmotic pressure of haemolymph	Females teneral day 1	13.3-23.6		
	mature day 1	16.1-26.2	5.15-7.98	1.14-3.39
	mature day 5	17.1-23.9	5.48-7.02	2.02-3.71
	Males teneral day 1	19.2-24.5		
	mature day 1	19.8-23.0	4.23-7.68	1.48-2.74
	mature day 5	14.6-23.8	4.20-7.25	1.49-2.84

Experiment	Trials		Range of emergence weights in mg	Range of mg blood imbibed all 3 meals/ mg emergence weight	Range of mg blood imbibed 3rd meal/mg emergence weight
Dilute blood meals	Meal size 3rd meal and subsequent weight loss	42%	16.2-26.3	see results	
		83%	13.1-25.2		
	Activity measurements	42%	16.2-26.3		
		83%	16.1-25.2		
Partial meals	PF2		13.4-26.3	2.95-4.92	0.131-0.779
	PF4		15.4-24.8	2.28-5.57	0.101-0.309
	NF2		17.7-26.1	3.12-4.56*	-
	NF4		13.9-24.3	3.0 -4.49*	-
Probing			12.2-26.3	3.4 -8.06	1.21 -3.13
Observations	Spontaneous activity in actograph		17.9-24.3	5.13-6.48	1.74-2.43
	in arena		18.9-23.2	5.27-7.49	1.48-2.76
	Visual stimulation offered		17.7-23.3	5.11-7.55	1.88-2.59

* Two meals only offered to these flies

APPENDIX II

1) Output from analysis of spontaneous activity, programme by A. Ludlow- activity burst shown in Fig. 9.4. underlined.

Fly 3 day 2
E52
NUMBER OF BOUTS 383
TOTAL TIME 16400.0
UNITS PER SEC 3.0
NUMBER OF RECOGNISED ACTIVITIES 5
CODES 44 06 05 84 04 00 00 00 00 00 00 00 00 00 00 00 00 00 00 00
ACTIVITIES
A CLEAN B WALK C PROBE
D FLIGHT E REST

SEQUENCE OF BOUTS

10.3	F	182.7	E	35.7	A	41.7	E	44.7	A	1.7	E	15.3	A	23.3	E				
2.3	D	2.3	E	.7	D	1.3	E	1.3	D	.3	E	3.3	D	5.0	E				
3.0	A	1.3	E	3.0	D	.3	E	1.0	D	1.7	E	1.0	B	.7	D				
.7	E	1.7	D	1.3	E	1.0	D	1.0	E	3.7	D	3.7	E	.3	B				
.3	E	1.3	D	1.7	E	.7	B	.3	E	1.7	D	2.3	E	1.3	D				
.7	E	1.3	D	.7	E	.3	B	1.3	E	2.0	D	.3	E	3.0	D				
1.3	E	.3	B	.3	E	.7	A	2.0	E	.3	D	.3	E	2.0	D				
2.7	E	1.3	D	4.0	E	.3	A	.3	E	1.3	D	2.7	E	9.3	A				
80.7	E	2.3	D	.3	E	1.7	D	2.0	E	10.7	A	166.7	E	2.7	A				
2.3	D	.3	E	1.0	D	2.3	E	2.3	D	2.0	E	3.3	D	.3	E				
3.7	D	5.0	E	11.7	A	146.0	E	2.0	D	1.0	E	2.0	D	2.3	E				
2.7	D	1.7	E	1.0	D	2.0	E	1.0	D	.7	E	.7	D	1.7	E				
1.0	A	.7	E	9.0	A	2.0	E	3.0	D	3.7	E	.7	D	.7	E				
1.0	D	1.0	E	1.3	D	1.7	E	5.0	A	.3	E	1.0	D	.3	E				
1.7	D	1.7	E	1.0	D	1.7	E	1.7	D	1.7	E	19.3	A	259.3	E				
1.3	D	.3	E	.3	B	3.3	D	2.7	E	1.0	D	2.7	E	1.0	D				
2.7	E	2.7	D	2.7	E	.7	D	2.3	E	3.0	D	2.3	E	4.0	A				
.3	E	1.0	D	.7	E	.3	B	.7	E	1.7	D	3.3	E	6.0	D				
679.7	E	1.0	D	.7	E	1.3	D	.3	E	4.3	D	.7	E	2.0	B				
27.3	A	5.3	E	126.0	A	6.3	E	2.0	D	.3	E	2.3	D	5.0	E				
2.7	D	3.7	E	3.3	D	.3	E	1.3	D	.3	E	5.7	D	.3	E				
1.0	D	110.7	E	68.3	A	132.7	E	1.3	D	.7	E	2.0	D	3.0	E				
2.3	D	2.3	E	.7	D	1.0	E	.3	B	1.0	E	3.7	D	5.3	E				
1.7	A	.7	E	3.3	D	.7	E	1.0	D	2.7	E	1.7	D	2.7	E				
1.0	D	1.0	E	1.3	B	2.7	D	1.0	E	1.0	D	1.7	E	1.3	D				
.7	E	1.3	D	2.3	E	1.7	D	2.0	E	2.0	D	4.0	E	1.0	D				
.3	E	1.7	D	3.0	E	8.0	A	.3	E	2.0	D	3.0	E	2.0	D				
.3	E	1.0	D	.7	E	.7	B	2.0	E	1.3	D	2.3	E	.7	D				
1.0	E	1.0	D	2.0	E	1.7	D	2.3	E	1.0	D	1.3	E	.3	B				
1.7	E	1.0	D	.3	E	1.0	D	1.0	E	2.0	D	9.0	E	1.3	D				
3.0	E	13.0	A	1102.0	E	2.0	D	.3	E	1.3	D	2.3	E	2.0	D				
2.3	E	1.3	D	2.0	E	1.7	D	2.0	E	1.0	B	.3	E	1.7	A				
.7	E	1.0	D	1.0	E	2.0	D	1.3	E	2.0	D	2.3	E	1.7	D				
1.7	E	2.0	D	.7	E	3.3	D	4.0	E	1.3	D	2.7	E	2.3	A				
.3	E	1.0	D	.3	E	.3	B	1.7	D	.7	B	.3	E	1.3	D				
.3	E	3.3	D	3.7	E	3.7	A	1.7	E	.3	D	1.7	E	1.3	D				
1.3	E	2.3	D	1.3	E	1.3	D	.3	E	.3	B	.3	E	3.7	A				
1.7	E	.7	D	.3	E	.3	B	629.0	E	1.3	D	.3	E	2.7	D				
4.0	E	1.0	D	1.0	E	3.0	D	2.3	E	.3	B	.7	D	3.3	E				
1.7	D	.3	E	2.3	D	5.0	E	2.3	D	1.0	E	2.3	D	2.0	E				
2.0	D	1.7	E	.7	D	1.0	E	2.0	D	4.7	E	.7	D	.3	E				
1.0	D	.7	E	.3	B	1.3	E	1.0	D	.3	E	.3	B	.3	E				
1.3	D	3.0	E	1.0	D	1.7	E	.7	D	.7	E	.7	B	1.0	E				
1.0	D	2.0	E	1.3	D	1.3	E	1.0	D	2.3	E	4.0	A	1.0	D				
.3	E	.7	B	1.7	E	.7	D	.7	E	.7	B	1.3	E	.7	D				
1.0	E	.3	B	.3	E	.7	D	1.0	E	1.7	D	.7	E	.7	D				
.3	B	.7	E	.3	D	2.7	E	1.0	D	.3	E	.3	B	3.0	E				
.7	D	.3	B	.7	D	.7	E	2.7	D	313.0	E	12.7	F						

1) Contd.

ANALYSIS OF ACTIVITY BURSTS

	CLEANING		WALKING		PROBING		FLIGHT		RESTING		LENGTH	INTERVAL
1	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	10.3	182.7
2	35.7	1	0.0	0	0.0	0	0.0	0	0.0	0	35.7	41.7
3	60.0	2	0.0	0	0.0	0	0.0	0	1.7	1	61.7	23.3
4	13.3	4	2.7	5	0.0	0	34.3	20	40.3	27	90.7	80.7
5	10.7	1	0.0	0	0.0	0	4.0	2	2.3	2	17.0	166.7
6	14.3	2	0.0	0	0.0	0	12.7	5	10.0	5	37.0	146.0
7	34.3	4	0.0	0	0.0	0	20.7	14	24.7	17	79.7	259.3
8	4.0	1	.7	2	0.0	0	21.7	10	20.7	11	47.0	679.7
9	153.3	2	2.0	1	0.0	0	25.0	10	23.3	11	203.7	110.7
10	68.3	1	0.0	0	0.0	0	0.0	0	0.0	0	68.3	732.7
11	22.7	3	2.7	4	0.0	0	45.7	29	66.3	34	137.3	1102.0
12	11.3	4	2.7	5	0.0	0	35.0	21	43.3	27	92.3	629.0
13	4.0	1	4.3	10	0.0	0	41.7	32	56.0	37	106.0	313.0
TOTAL TIME	432.0		15.0		0.0		240.7		288.7			
NUMBER	26		27		0		143		172			

TOTAL ACTIVITY INCLUDING RESTS

TOTAL TIME	432.0		15.0		0.0		240.7		4756.0			
NUMBER	26		27		0		143		185			

TOTAL HEADER AND TRAILER 23. 23.

2) Programme by the author to calculate mean bout length per activity burst, and its output.

```
00100 SUBROUTINE LENGTH
00110C
00120C FINDS AVERAGE LENGTH CLEANP, CLEANB, CLEANA, FLIGHT,
00130C WALK, RESTB, FOR EACH BURST.
00140C
00150 COMMON TITLE(8),ACT(21),BLENTH(4000),PERSEC,CLOCK,
00160+ NACT,NBOUT,IBOUT(4000),ICHAR(21)
00170 DIMENSION A(30,6),N(30,6),AV(30,6)
00180 DO 1 IJ=1,30
00190 DO 1 IN=1,6
00200 1 AV(IJ,IN)=0.0
00210 DO 2 JZ=1,30
00220 DO 2 NZ=1,6
00230 2 A(JZ,NZ)=0.0
00240 DO 3 JY=1,30
00250 DO 3 NY=1,6
00260 3 N(JY,NY)=0
00270 J=0
00280 K=1
00290 I=0
00300 WRITE(6,10)
00310 10 FORMAT(1H1,36HAVERAGE LENGTH OF EACH ACT PER BURST)
00320 20 I=I+1
00330 IF(I.GT.NBOUT)GO TO 777
00340 IF(IBOUT(I).EQ.6)GO TO 20
00350 IF(IBOUT(I).NE.5)GO TO 40
00360 IF(BLENTH(I).GT.20)GO TO 500
00370 40 GO TO (50,60,20,80,90),IBOUT(I)
00380C
00390C  DIFFERENTIATES THE THREE CONTEXTS OF CLEANING
00400C
00410 50 IF(BLENTH(I-1).GT.20)GO TO 54
00420 IF(BLENTH(I+1).GT.20)GO TO 52
00430 N(K,1)=N(K,1)+1
00440 A(K,1)=A(K,1)+BLENTH(I)
00450 GO TO 20
00460 52 N(K,2)=N(K,2)+1
00470 A(K,2)=A(K,2)+BLENTH(I)
00480 GO TO 20
00490 54 IF(BLENTH(I+1).GT.20)GO TO 56
00500 N(K,2)=N(K,2)+1
00510 A(K,2)=A(K,2)+BLENTH(I)
00520 GO TO 20
00530 56 N(K,3)=N(K,3)+1
00540 A(K,3)=A(K,3)+BLENTH(I)
00550 GO TO 20
00560C
00570C  ACCUMULATES LENGTHS AND NUMBERS OF REMAINING ACTS
00580C
00590 60 N(K,4)=N(K,4)+1
00600 A(K,4)=A(K,4)+BLENTH(I)
00610 GO TO 20
00620 80 N(K,5)=N(K,5)+1
00630 A(K,5)=A(K,5)+BLENTH(I)
00640 GO TO 20
00650 90 N(K,6)=N(K,6)+1
00660 A(K,6)=A(K,6)+BLENTH(I)
00670 GO TO 20
00680 500 J=J+1
00690C
00700C WORKS OUT AVERAGE LENGTHS
00710C
00720 DO 100 L=1,6
00730 100 AV(K,L)=A(K,L)/N(K,L)
00740 K=K+1
00750 GO TO 20
00760 777 WRITE(6,800)
00770 800 FORMAT(1X,2X,39HCLEANB CLEANP CLEANA WALK   FLIGHT REST)
00780 WRITE(6,850)((AV(K,L),L=1,6),K=1,J)
00790 850 FORMAT(1X,6(F6.1,1X))
00800C
00810C
00820 RETURN
00830 END
```

2) Contd.

Fly 3 day 2

AVERAGE LENGTH OF EACH ACT PER BURST

CLEANB	CLEANP	CLEANA	WALK	FLIGHT	REST
1	1	35.7	1	1	1
1	30.0	1	1	1	1.7
1.3	9.3	1	.5	1.7	1.5
1	10.7	1	1	2.0	1.2
1	7.2	1	1	2.5	2.0
5.0	19.3	1	1	1.5	1.5
4.0	1	1	.3	2.2	1.9
76.7	1	1	2.0	2.5	2.1
1	1	68.3	1	1	1
4.8	13.0	1	.7	1.6	2.0
2.8	1	1	.5	1.7	1.6
4.0	1	1	.4	1.3	1.5

3) Programme by the author to analyse the data of spontaneous activity alternated with periods of visual stimulation, and its output.

```
   00100 SUBROUTINE PEN10
00110C
00115C    DIVIDES ACTIVITIES UNDER VARIOUS CIRCUMSTANCES
00120C   INTO BURSTS
00121C
00122C
00130C LENGTHS AND NUMBERS OF SESSION TOTALS
00132C
00140 COMMON TITLE(8),ACT(21),BLENTH(4000),PERSEC,CLOCK,
00150+ NACT,NBOUT,IBOUT(4000),ICHAR(21)
00160 DIMENSION A(30,15),N(30,15),AV(30,14),AT(12),NT(12)
00170 DO 1 M=1,12
00180 1 AT(M)=0.0
00190 DO 2 M=1,12
00200 2 NT(M)=0.0
00410 J=0
00420 K=1
00430 I=0
00432 DO 921 IJ=1,30
00434 DO 921 IN=1,14
00436 921 AV(IJ,IN)=0.0
00440 WRITE(6,10)
00450 10 FORMAT(1H1,11X,11HSPONTANEOUS,26X,7HVISIBLE,34X,9HINVISIBLE)
00460 WRITE(6,20)
00470 20 FORMAT(1X,6X,5HCLEAN,5X,4HWALK,6X,6HFLIGHT,4X,4HREST,7X,
00480+5HCLEAN,5X,4HWALK,6X,6HFLIGHT,4X,4HREST,8X,5HCLEAN,5X,
00490+4HWALK,6X,6HFLIGHT,3X,4HREST,2X,8HINTERVAL)
00500C
00510C LENGTHS AND NUMBERS OF BURST TOTALS
00520C
```

3) Contd.

```
00530 30 CONTINUE
00540 B=0.0
00550 DO 66 JZ=1,30
00560 DO 66 NZ=1,15
00570 66 A(JZ,NZ)=0
00580 DO 77 JY=1,30
00590 DO 77 NY=1,15
00600 77 N(JY,NY)=0
00610 QUEST=0.0
00620 IQUEST=0
00630 BTOT=0.0
00640 40 I=I+1
00650 IF(I.GT.NBOUT)GO TO 777
00660 IF(IBOUT(I).EQ.11)GO TO 40
00670 IF(IBOUT(I).NE.5)GO TO 60
00680C
00690C DEFINES END OF BURST
00700C
00710 IF(BLENTH(I).GT.20)GO TO 910
00720 60 IF(IBOUT(I).LE.5)GO TO 699
00730C
00740C SORTS STIMULATED ACTS INTO STRIPE VISIBLE OR INVISIBLE, AND ADDS UP
00750C BURST TOTALS
00760C
00762C  ORDER= CLEAN WALK FLIGHT REST
00763C
00770 65 IF(B.NE.0.0)GO TO 70
00780 68 GO TO(40,40,40,40,40,81,80,40,83,84),IBOUT(I)
00790 80 N(K,1)=N(K,1)+1
00800 A(K,1)=A(K,1)+BLENTH(I)
00810 GO TO 71
00820 81 N(K,2)=N(K,2)+1
00830 A(K,2)=A(K,2)+BLENTH(I)
00840 GO TO 71
00850 83 N(K,3)=N(K,3)+1
00860 A(K,3)=A(K,3)+BLENTH(I)
00870 GO TO 71
00880 84 N(K,4)=N(K,4)+1
00890 A(K,4)=A(K,4)+BLENTH(I)
00900 71 B=B+1.0
00910 GO TO 72
00920 70 GO TO(40,40,40,40,40,86,85,40,87,88),IBOUT(I)
```

3)Contd.

```
00930 85 N(K,5)=N(K,5)+1
00940 A(K,5)=A(K,5)+BLENTH(I)
00950 GO TO 72
00960 86 N(K,6)=N(K,6)+1
00970 A(K,6)=A(K,6)+BLENTH(I)
00980 GO TO 72
00990 87 N(K,7)=N(K,7)+1
01000 A(K,7)=A(K,7)+BLENTH(I)
01010 GO TO 72
01020 88 N(K,8)=N(K,8)+1
01030 A(K,8)=A(K,8)+BLENTH(I)
01040 72 BTOT=BTOT+BLENTH(I)
01050 QUEST=BTOT/8.5
01060 IQUEST=QUEST
01070 IF(IQUEST.LT.1)B=0.0
01080 IL=2
01090 DO 62 IL=2,10,2
01100 IF(IQUEST.EQ.IL)B=0.0
01110 62 CONTINUE
01120 GO TO 900
01130C
01140C ACCUMULATES SPONTANEOUS NUMBER AND LENGTH OF ACTS FOR EACH BURST
01150C
01153C STARTS NEW BURST IF AROUSAL FROM STRIPE CONTINUES
01154C
01155 699 IF(5.LT.IBOUT(I-1).AND.IBOUT(I-1).LT.11)GO TO 910
01160 700 GO TO(94,93,40,95,96),IBOUT(I)
01170 93 N(K,9)=N(K,9)+1
01180 A(K,9)=A(K,9)+BLENTH(I)
01190 GO TO 40
01193C
01194C DIFFERENTIATES THE THREE CONTEXTS OF CLEANING
01195C
01200 94 IF(BLENTH(I-1).GT.20)GO TO 105
01210 IF(BLENTH(I+1).GT.20)GO TO 100
01220 N(K,13)=N(K,13)+1
01230 A(K,13)=A(K,13)+BLENTH(I)
01235 GO TO 40
01240 100 N(K,14)=N(K,14)+1
01250 A(K,14)=A(K,14)+BLENTH(I)
01255 GO TO 40
01260 105 IF(BLENTH(I+1).GT.20)GO TO 106
```

3)Contd.

```
01270 N(K,14)=N(K,14)+1
01280 A(K,14)=A(K,14)+BLENTH(I)
01285 GO TO 40
01290 106 N(K,15)=N(K,15)+1
01300 A(K,15)=A(K,15)+BLENTH(I)
01310 GO TO 40
01320 95 N(K,11)=N(K,11)+1
01330 A(K,11)=A(K,11)+BLENTH(I)
01340 GO TO 40
01350 96 N(K,12)=N(K,12)+1
01360 A(K,12)=A(K,12)+BLENTH(I)
01370 900 GO TO 40
01380C
01390C WRITES BURSTS AND TOTALS FOR EACH ACTIVITY
01400C
01410 910 J=J+1
01420 N(K,10)=N(K,10)+N(K,13)+N(K,14)+N(K,15)
01430 A(K,10)=A(K,10)+A(K,13)+A(K,14)+A(K,15)
01440 WRITE(6,920)J,A(K,10),N(K,10),A(K,9),N(K,9),A(K,11),N(K,11),
01450+ A(K,12),N(K,12),A(K,2),N(K,2),A(K,1),N(K,1),A(K,3),N(K,3),A(K,4),
01460+ N(K,4),A(K,6),N(K,6),A(K,5),N(K,5),A(K,7),N(K,7),A(K,8),N(K,8),
01470+ BLENTH(I)
01480 920 FORMAT(1X,I2,1X,4(F6.1,1X,I2,1X)1X,4(F6.1,1X,I2,1X)
01490+ 1X,4(F6.1,1X,I2,1X),1X,F6.1)
01500 DO 923 M=1,12
01510 923 AT(M)=AT(M)+A(K,M)
01520 DO 922 M=1,12
01530 922 NT(M)=NT(M)+N(K,M)
01540C
01550C WORKS OUT AVERAGE LENGTHS FOR EACH BURST
01560C
01570 DO 980 L=1,9
01580 980 AV(K,L)=A(K,L)/N(K,L)
01590 LM=10
01600 DO 981 L=13,15
01610 AV(K,LM)=A(K,L)/N(K,L)
01620 981 LM=LM+1
01630 LM=13
01640 DO 982 L=11,12
01650 AV(K,LM)=A(K,L)/N(K,L)
01660 982 LM=LM+1
01880C
```

3) Contd.

```
01890C
01900 K=K+1
01910 GO TO 30
01920 777 WRITE(6,924)
01930 924 FORMAT(1X,6HTOTALS)
01940 WRITE(6,925)AT(10),AT(9),AT(11),AT(12),AT(2),AT(1),AT(3),
01945+ AT(4),AT(6),AT(5),AT(7),AT(8)
01950 925 FORMAT(1X,3X,4(F6.1,4X)2X,4(F6.1,4X)2X,4(F6.1,4X))
01960 WRITE(6,926)NT(10),NT(9),NT(11),NT(12),NT(2),NT(1),NT(3),
01965+ NT(4),NT(6),NT(5),NT(7),NT(8)
01970 926 FORMAT(1X,7X,4(I2,8X)2X,4(I2,8X)2X,4(I2,8X))
01990C
02000 WRITE(6,1015)
02010 1015 FORMAT(1H1,37HAVERAGE LENGTHS OF EACH ACT UNDER THE
02020+30H THREE DIFFERENT CIRCUMSTANCES)
02021C
02022C  AVERAGE ORDER IS WALK CLEAN FLIGHT REST
02023C
02030 WRITE(6,1018)
02040 1018 FORMAT(1X,7HVISIBLE/1X,2X,23HWALK  CLEAN FLIGHT REST)
02060 WRITE(6,1020)((AV(K,L),L=1,4),K=1,J)
02063 WRITE(6,1023)
02064 1023 FORMAT(1X,9HINVISIBLE/1X,2X,23HWALK  CLEAN FLIGHT REST)
02065 1020 FORMAT(1X,4(F5.1,1X))
02066 WRITE(6,1021)((AV(K,L),L=5,8),K=1,J)
02068 1021 FORMAT(1X,4(F5.1,1X))
02069 WRITE(6,1027)
02070 1027 FORMAT(1X,11HSPONTANEOUS)
02071 WRITE(6,1024)
02072 1024 FORMAT(1X,2X,39HWALK   CLEANB CLEANP CLEANA FLIGHT REST)
02075 WRITE(6,1026)((AV(K,L),L=9,14),K=1,J)
02080 1026 FORMAT(1X,6(F6.1,1X))
02090C
02100C
02110 RETURN
02120 END
02130C
02140C
02150C
/
```

3) Contd.

S42

NUMBER OF BOUTS 135
TOTAL TIME 8560.0
UNITS PER SEC 3.0
NUMBER OF RECOGNISED ACTIVITIES 10
CODES 04 02 01 08 10 84 82 81 88 90 00 00 00 00 00 00 00 00 00 00
ACTIVITIES

A CLEAN B WALK C PROBE

D FLIGHT E REST F SCLEAN

G SWALK H SPROBE I SFLIGHT

J SREST

SEQUENCE OF BOUTS

49.0	K	283.0	E	38.3	A	472.3	E	1.0	D	4.0	E	2.7	D	3.7	E
1.0	D	.3	E	1.3	D	2.3	E	3.3	D	1.7	E	2.7	D	2.7	E
5.7	D	3.3	E	2.0	D	2.0	E	5.7	D	4.3	E	5.3	D	7.7	E
3.0	D	4.7	E	5.3	A	521.3	E	15.7	A	2.7	D	1.0	E	4.7	D
3.3	E	2.0	D	2.0	E	2.3	D	2.3	E	7.3	D	4.3	E	4.7	D
2.7	E	3.0	D	2.0	E	4.0	D	3.3	E	5.0	D	15.0	E	4.7	J
1.7	I	11.7	J	2.0	I	1.3	J	4.0	I	11.7	J	3.0	I	3.7	J
4.7	I	12.0	J	1.3	I	30.3	J	276.0	E	20.3	A	201.7	E	103.7	A
118.3	E	4.3	D	2.7	E	1.0	D	.7	E	1.7	D	2.3	E	1.7	D
1.3	E	3.0	D	5.0	E	3.0	D	2.0	E	1.7	D	1.0	E	1.3	D
1.0	E	3.0	D	2.0	E	2.3	D	2.7	E	2.0	D	3.3	E	8.7	A
3.0	D	2.7	E	.3	B	1.3	D	2.0	E	1.0	D	.7	E	2.0	D
3.0	E	3.3	A	.3	D	1.7	E	2.3	D	3.3	E	3.0	D	4.0	E
5.3	D	2.7	E	15.0	A	210.3	E	6.0	J	1.0	I	.3	J	1.7	I
1.3	J	1.3	I	9.7	J	1.0	I	.3	J	1.0	I	1.7	J	2.7	I
2.3	J	3.3	I	.7	J	.7	G	4.7	J	2.7	I	10.3	J	1.0	I
38.7	J	.7	E	1.0	D	.7	E	1.7	D	56.0	E	37.0	K		

3)Contd.

SPONTANEOUS

	CLEAN		WALK		FLIGHT		REST	
1	0.0	0	0.0	0	0.0	0	0.0	0
2	38.3	1	0.0	0	0.0	0	0.0	0
3	5.3	1	0.0	0	33.7	11	36.7	11
4	15.7	1	0.0	0	35.7	9	36.0	9
5	20.3	1	0.0	0	0.0	0	0.0	0
6	103.7	1	0.0	0	0.0	0	0.0	0
7	27.0	3	.3	1	43.3	19	44.0	19
8	0.0	0	0.0	0	0.0	0	0.0	0
9	0.0	0	0.0	0	2.7	2	.7	1
TOTALS								
	210.3		.3		115.3		117.3	
	8		1		41		40	

VISIBLE

	CLEAN		WALK		FLIGHT		REST	
1	0.0	0	0.0	0	0.0	0	0.0	0
2	0.0	0	0.0	0	0.0	0	0.0	0
3	0.0	0	0.0	0	0.0	0	0.0	0
4	0.0	0	0.0	0	10.7	4	33.0	5
5	0.0	0	0.0	0	0.0	0	0.0	0
6	0.0	0	0.0	0	0.0	0	0.0	0
7	0.0	0	0.0	0	0.0	0	0.0	0
8	0.0	0	.7	1	11.0	7	62.0	7
9	0.0	0	0.0	0	0.0	0	0.0	0
TOTALS								
	0.0		.7		21.7		95.0	
	0		1		11		12	

INVISIBLE

	CLEAN		WALK		FLIGHT		REST		INTERVAL
1	0.0	0	0.0	0	0.0	0	0.0	0	283.0
2	0.0	0	0.0	0	0.0	0	0.0	0	472.3
3	0.0	0	0.0	0	0.0	0	0.0	0	521.3
4	0.0	0	0.0	0	6.0	2	42.3	2	276.0
5	0.0	0	0.0	0	0.0	0	0.0	0	201.7
6	0.0	0	0.0	0	0.0	0	0.0	0	118.3
7	0.0	0	0.0	0	0.0	0	0.0	0	210.3
8	0.0	0	0.0	0	4.7	2	14.0	4	.7
9	0.0	0	0.0	0	0.0	0	0.0	0	56.0
TOTALS									
	0.0		0.0		10.7		56.3		
	0		0		4		6		