STUDIES ON THE WATER RELATIONS OF ADULT LOCUSTS — III
THE WATER BALANCE OF NON-FLYING LOCUSTS

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ABSTRACT

The object of this work was to draw up a balance sheet of water gains and losses in non-flying adult *Locusta*. Metabolic water is produced at the rate of 0,6 mg/locust/h and is insufficient to balance transpiratory losses (10,3 mg/locust/h in dry air). Adult locusts are unable to absorb water vapour from air of up to 97,5% R.H., nor are they able to absorb liquid water through the body surface or anus. They do, however, drink and can gain as much as 120 mg water/g weight in 30 min. It is shown that desiccation or exsanguination elicit a strong drinking response. Food is shown to be the most important source of water – 51,3 mg/locust/h if the food is fresh grass and losses in the faeces in this case amount to 30,7 mg/locust/h. If fed on dried grass the locust gains only 0,2 mg/locust/h, partly because of feeding inhibition when only dry food is available. At the same time faecal water loss is 1,6 mg/locust/h and taken with transpiratory water loss leads to a substantial deficit. Transpiratory water loss is discussed and evidence given for the reduction in ventilation of *Locusta*, *Schistocerca* and *Chortoicetes* when severely desiccated by feeding on dry food. Survival under conditions of non-replenishment of food and water reserves was assessed. *Locusta* starved at 91% R.H. died after an average of 189h when their fat reserves were reduced to 6,6% (live weight basis) but had normal water content. *Locusta* starved at 0% R.H. died after an average of 134h when the water content was reduced to 55%, the fat content still being 9,1%. It is shown that 20% of the water in normally hydrated locusts is present in the blood, and most of this appears to be available as a water reserve. Estimates of the fat consumption of starving locusts (0,26 mg/g/h) show good agreement with the fat consumption calculated from oxygen consumption data. The water balance sheet clearly indicates that, in feeding locusts, the gain of water in the food and loss in faeces are the most important avenues of gain and loss. Regulation of water loss in the faeces is discussed.

INTRODUCTION

Although considerable information concerning the various physiological systems which maintain the water balance of locusts is available (Uvarov 1966), the water relations of the adult locust have received relatively little attention. While water balance sheets for the larva of *Tenebrio*, for the blood-sucking bug, *Rhodnius*, and perhaps for the cockroach, *Periplaneta*, can be drawn up from studies by various authors, only in one case, that of *Glossina* pupae and adults, has a comprehensive study under the same standardized conditions and giving quantitative data been completed (Bursell 1958; 1959). There is a considerable amount of work on the water relations of *Locusta* and grasshopper eggs (e.g. Matthée 1951) and their nymphal stages (e.g. Blackith 1961). In the present paper an attempt is made to evaluate the balance between water gain and loss of the adult African migratory locust, *Locusta migratoria migratorioides* (Reiche & Fairmaire), and to determine how the animal regulates its water balance according to its needs. Some observations on adult *Chortoicetes terminifera* (Walker) and *Schistocerca gregaria* (Forskål) are also included for comparative purposes.

The success of locusts as migratory insects, living very often in arid areas unfavourable for the maintenance of water balance, may be attributed to their comparatively large size and water reserves. The gain and loss of water from an insect occurs in many ways (Buxton 1932a), and to ensure a relatively constant "milieu interieur" contribution of gain and loss must be controlled, so that one does not greatly exceed the other. The factors affecting water balance are: gain of water with the food, by breakdown of metabolites, by drinking and by absorption from the air or from liquid water; loss in the faeces, from the tracheal system and through the cuticle. Food is the main source of water gain; water availability in food may be as high as 85–95% in plant sap, 70–90% in green plants, about 79% in blood, down to 5% or less in dried vegetation or stored foods. Thus, insects may have to excrete or conserve water to maintain water balance.

That the lipid epicuticle is the barrier to the outward diffusion of water has been recognized for many years, but only recently have the properties and many of the water relations of the cuticle been elucidated (Beament 1961). The existence of valves guarding the openings to the tracheal system of most terrestrial insects is, by implication, a powerful means of reducing water losses. The demand for oxygen in an active terrestrial animal must be reconciled with the need to prevent excessive water losses. The respiratory system must be kept moist and in intimate contact with the active tissues, or respiratory gas exchange would not occur. Since water vapour is also a gas, its movement will be dependent on its concentration in the tracheal system relative to the outside air, and usually this movement would be outwards from the animal. Where the insect's body temperature is above that of the air, the gradient for water losses would exist even in saturated air. Since oxygen is required in increased quantity during periods of activity, the spiracles must remain open longer, resulting in increased rates of water loss (Bursell 1957). Increased water loss accompanies evaporative cooling during periods of heat stress (Edney 1957), and when the spiracles are kept open experimentally using CO_2 (Mellanby 1934). Discontinuous patterns of respiration are thought to be a sophisticated mechanism of water conservation (Buck 1958). Koidsumi (1934–35), Ramsay (1935) and numerous other workers have attempted to measure the relative contributions of water loss from spiracles and cuticle.

Wigglesworth (1932) found that the rectal glands were able to remove a high proportion of water from the food. More recent work (Phillips 1964) has shown that water resorption from the rectum of some insects is an active process taking place against a concentration gradient. Rectal reabsorption, super-imposed on the excretion of large proportions of nitrogenous waste as insoluble uric acid, means that considerable amounts of water can be conserved. The role of the excretory system in the maintenance of water and osmotic balance has recently been reviewed by Stobbart & Shaw (1964).

During respiration, food and food reserves are broken down, and the breakdown products of oxidative catabolism usually include a certain amount of water. This is the so-called "metabolic water", the contribution of which to water balance in insects has long been a matter of controversy. Metabolic water as defined here does not refer to unbound water taken in with the food, but only to that formed when hydrocarbons are completely oxidized. Sources of free water are sometimes available to insects to supplement that taken in with the food. These sources include drinking, absorption of water vapour from non-saturated air and absorption of contact water through the body surface or through specialized cuticular areas (Edney 1957).

MATERIALS AND METHODS

Locusta migratoria migratorioides (R. & F.), African migratory locust, were reared in an insectary (29,5 ± 0,5°C; 16 h light in 24 h) in Salisbury as previously described (Loveridge 1973). *Schistocerca gregaria* (Forsk.), the desert locust, and *Chortoicetes terminifera* (Walk.), the Australian plague locust, were reared in the insectaries of the Anti-Locust Research Centre (now Centre for Overseas Pest Research). These three species are referred to hereafter as *Locusta*, *Schistocerca* and *Chortoicetes*. All experiments on *Locusta* were done at a temperature of 29,5 ± 0,5°C; experiments with *Chortoicetes* and *Schistocerca* at the Anti-Locust Research Centre during 1967 were at a basal temperature of 26,0°C, rising during the day (when cage lights were on) to 30–31°C. Dead locusts were dried and their body fat extracted using methods described elsewhere (Loveridge 1973). Dry weight and residual dry weight (R.D.W.) or fat free dry weight were thus obtained. Faeces were dried by storing over P_2O_5 for 24 h before weighing to 0,1 mg. Locusts were exsanguinated in certain experiments (or to estimate the blood volume) by cutting a small hole in the frons and gently centrifuging in a hand centrifuge while head downward in a tapered tube (Sternburg & Corrigan 1959). The amount of water in the blood was estimated by evaporating samples to dryness and weighing the remaining solids. To provide a picture of the water balance of the locust, the data were converted to a "standard locust" of 1,6 g live weight. In the case of cuticular and spiracular water loss the calculated regression of water loss on weight was used; in other experiments mean rates of gain or loss were adjusted by simple proportion. All error estimates given in this paper are ± twice the standard error of the mean.

Other methods in the various experiments are described at the appropriate place in the text.

METABOLIC WATER

Work on certain stored-products insects (Fraenkel & Blewett 1944) has established that larvae consume more food at low humidities, utilizing a proportion of the food consumed to produce metabolic water. Thus they were able to show how larvae reared in food containing as little as 1% water contained 64% body water on pupation. It has subsequently often been assumed that the regulation of production of water from food or stored food reserves might account for the survival of insects in dry environments (e.g. Barton-Browne 1964). The regulation of metabolic water – in the sense that more can be produced under desiccating conditions by increasing the metabolic rate – has yet to be shown experimentally. The theory has been criticized by Mellanby (1942) who argued that increased metabolism would require more oxygen, with resultant increased water loss through the spiracles.

It has been shown (Loveridge & Bursell in press) that adult *Locusta* do not increase their metabolic rate after desiccation for 24–48 h. The metabolic rate remains the same after a variety of starvation treatments and various dietary regimes. The respiratory quotient, however, was lowered following starvation, indicating a metabolism geared more to fats than to carbohydrates. It was calculated that the metabolic water production of *Locusta* at 30°C was 0,4 mg/g/h when fed and 0,3 mg/g/h when starved (Loveridge & Bursell in press).

WATER GAIN WITH FOOD, LOSS IN FAECES

This aspect of the water balance of locusts has been reported elsewhere (Loveridge in press). When feeding on fresh grass containing 85% water, locusts produce faecal pellets containing 80% water. This allows them to eliminate excess water; the figures of percentage water in themselves are misleading as the food is substantially reduced in dry weight due to digestion. By contrast, if fed on dried food (containing less than 10% water) the locusts are unable to remain in water balance. They produce faeces of 20–30% water content but reduce food intake, lose weight and often resort to cannibalism to obtain water. Yet locusts fed on the same dry food and given water to drink feed and grow normally. Gain in the food and loss in the faeces can constitute the major part of water gain and loss. It is not surprising, therefore, that the control of water balance by malpighian tubule and rectal function has been the subject of recent research (Shaw & Stobbart 1972).

FREE WATER SOURCES AND GAIN FROM THE ATMOSPHERE

Drinking

Observations made during the present work indicated that drinking from water-soaked cotton wool occurred in the laboratory colony of locusts. The experiment reported below provides an estimate of the amounts of water taken in. That drinking of water is important when only dry food is available has been demonstrated (Loveridge in press). It is difficult to assess how important drinking is in maintaining water balance in the field. The possibility that drinking of dew or of water from permanent pools during the dry season takes place in field populations cannot be excluded.

Male and female *Locusta* of age between 11 and 20 days from fledging were used in these experiments. After pre-treatment they were allowed to drink on a large pad of cotton wool soaked with tap water. The locusts were never observed to eat the cotton wool, and dissection of locusts which had increased in weight after drinking on the cotton wool showed that no cotton wool fibres were present in the gut (cf. the experiments of Husain & Mathur 1936; Loveridge in press). Pre-treatments (in order of increasing desiccation) were as follows:

(i) 5 ♂♂, 5 ♀♀ removed from cage after feeding normally for 4 hours.
(ii) 5 ♂♂, 5 ♀♀ starved for 24 hours at 96% R.H.
(iii) 5 ♂♂, 5 ♀♀ starved for 24 hours at 0% R.H.
(iv) 5 ♂♂, 5 ♀♀ starved for 48 hours at 0% R.H.
(v) 5 ♂♂, 5 ♀♀ starved for 24 hours at 96% R.H. and exsanguinated after weighing. After being allowed 3 h for recovery, they were weighed and allowed to drink. Mean weight of blood extracted was 227 mg.
(vi) 5 ♂♂, 5 ♀♀ fed on dry bran and grass (water content about 10%) for five days, and the same group after eight days.

The amount of water drunk by the locusts was estimated by weighing before being placed on the cotton wool pad and reweighing after half an hour. Locusts which drank did so imme-

TABLE 1

Amounts of water drunk by locusts after different pretreatments (see text). Figures represent weight gains (or losses) in mg/g over 30 minutes. The fiducial limits are ± 2 × S.E.

Treatment (*i*)	(*ii*)	(*iii*)	(*iv*)	(*v*)	(*vi*)	
fed fresh food	*starved 24h 96% R.H.*	*starved 24h 0% R.H.*	*starved 48h 0% R.H.*	*exsanguinated*	*fed dry food 5 days*	*8 days*
−9,4 mg	−1,5	+11,1	+68,7	+32,9	+134,2	+5,2
−1,8 mg	−1,9	+60,5	+30,4	+80,5	+87,3	—
−10,1 mg	−1,7	+17,2	+29,4	+79,1	+85,3	+39,6
−2,2 mg	−1,9	+43,6	+34,5	+43,5	+155,7	+156,7
−5,6 mg	−1,3	+46,9	+9,3	+51,4	+139,6	+180,1
−1,8 mg	−1,2	+37,6	+96,6	+80,7	+123,5	—
+10,5 mg	−1,9	+19,1	+24,3	+65,8	+135,5	+107,8
−13,8 mg	−0,7	+60,9	+11,4	+108,2	+58,7	+8,7
−11,6 mg	−1,1	+56,0	+5,6	+56,1	+161,6	+165,0
−0,8 mg	−1,0	+57,8	+83,6	+90,8		
7 faecal pellets: 51,1 mg ∴ net gain 4,5 mg = 0,24 mg/g	Loss 1,42 ± 0,27 mg/g	Gain 41,07 ± 12,07 mg/g	Gain 39,38 ± 20,38 mg/g	Gain 68,90 ± 14,64 mg/g	Gain 120,16 ± 23,42 mg/g	Gain 94,73 ± 57,48 mg/g

diately after being allowed access to the soaked cotton wool. After "testing" with antennae and palps, they "drank" by chewing the cotton wool between the mandibles, and presumably expressing the water into the cibarium.

The amounts of water drunk by locusts in the six treatments are given in Table 1. This clearly indicates that desiccation is the primary factor inducing locusts to drink. Locusts starved at high humidity, or fed locusts, drank infrequently, whereas locusts starved at low R.H. drank frequently. It should be noted that group (i) produced some faeces; if faecal weight loss is allowed for, an average of 0,24 mg/g water was drunk. Comparison of exsanguinated locusts (v) and locusts with normal blood volume and the same pre-treatment (ii) showed that blood volume probably played some part in causing locusts to drink. This evidence was reinforced by the data on the locusts fed on dry food. Observations on blood volumes of locusts with this treatment (Loveridge in press) showed that very little haemolymph was present. Locusts fed on dry food drank the most water (about 100 mg/g), exsanguinated locusts about 70 mg/g, and desiccated locusts about 40 mg/g.

The evidence presented shows that locusts are able to drink. They drink only under conditions where they need water, and the stimulus to drink may well be initiated by a reduced blood volume (Dethier & Evans 1961), which may be a sensitive indicator of the extent of water reserves.

Absorption of liquid water

The possibility that absorption of contact water might occur in the locust was tested in the following way. Six adult *Locusta*, about 12 days after fledging, were starved for 48 hours at 0% R.H. Their mouthparts were carefully blocked with soft paraffin wax, and after weighing they were placed on a cotton wool pad soaked with water. They were left for an hour on the pad, and reweighed. Weight changes are shown in Table 2. In only one out of six locusts was a gain observed. It is considered unlikely that this gain in weight was due to absorption, but to water droplets adhering to the animal. The locusts "searched" with their antennae, and applied their blocked mouthparts to the pad. When the wax was removed and locusts returned to their breeding cage, no fatalities were observed in the 24 hours following.

It is considered that *Locusta* is unable to absorb liquid water through the integument, and so-called anal drinking (Edney 1957) is also excluded.

Absorption of water vapour

An experiment was designed to determine whether *Locusta* adults were able to absorb water vapour. Fifteen male locusts of age 34 days after fledging were used in these experiments. They were weighed, divided into three groups of five each, and placed individually without food in 100 × 40 mm tubes covered with nylon gauze. The groups were placed separately in large desiccators in which relative humidity was controlled using saturated solutions of salts or P_2O_5 (Winston & Bates 1960). Temperature was controlled at 29,5 ± 0,5°C in the constant-temperature room in which the desiccators were kept. Two of the groups were kept at 0% R.H. until the third day when group 2 was removed to 97,5% R.H., group 1 remaining at 0% R.H. Group 3 was kept at 91% R.H. All the locusts were weighed daily, and any faeces produced were removed at the same time.

TABLE 2

Weight changes of locusts with blocked mouthparts allowed access to water-saturated cotton-wool 29,5°C.

Sex	*Weight (g)*	*Weight gain or loss after 1 hour (mg water/g live weight)*
♂	1,75	−2,2
♀	1,90	−2,2
♀	1,72	−2,0
♂	1,49	−1,6
♂	1,41	−2,1
♂	1,50	+1,1
		Mean loss = 1,50 ± 1,06 mg/g

TABLE 3

Mean weight losses of groups of five male locusts starved at 29,5 ± 0,5°C and held at various relative humidities

	Weight loss in mg/g body weight/hour		
	Group 1 0% R.H.	*Group 2 0%→97,5% R.H. on Day 3*	*Group 3 91% R.H.*
1st Day	3,9	—	1,1
2nd Day	3,3	—	1,0
3rd Day	3,1	—	1,0
4th Day	2,8	1,4	1,7
5th Day	2,4	0,7	1,2
6th Day	2,4	1,0	1,4
7th Day	All dead	1,3	2,4

The results of this experiment are summarized in Table 3 and representative examples are given in Figure 1.

Group I showed steady weight losses until death, although there seemed to be a decrease in rate which probably represented regulation of excretory and spiracular water losses. Group 2 showed a decrease in the rate of weight loss after being placed in the high relative humidity, but in no case was a gain in weight recorded, either at 6 hours, at 24 hours, or at any subsequent time after exposure to 97,5% R.H. Group 3 showed steady weight losses throughout. The animals of Group 2 lost weight at a lower rate from the 4th to 7th days than Group 3 (Table 3). Again this probably represented conservation of endangered water reserves after three days desiccation. It is concluded from this experiment that adult *Locusta* males are unable to absorb water from air of high relative humidity.

Drinking of water by insects has been reviewed by Edney(1957)and Bursell(1964). Barton-Browne (1964) has reviewed drinking in adult Diptera and Mellanby & French (1958) measured the amount of water drunk by larval insects of a variety of habitats and feeding habits. They found that the development of *Tenebrio* larvae was completed faster when drinking water was available, and that *Diataraxia* larvae drank enough water to restore their normal water content.

Drinking in Orthoptera (Acrididae) is either not well-known, or poorly investigated. Nikol'skiĭ (1925, quoted by Uvarov 1966) reported hoppers of *Locusta* drinking at pools in Middle Asia. Albrecht (1953) reported that 2 000 adult *Nomadacris* collected in the field in the Rukwa valley (Tanzania) died within 10 days when kept in cages. His observations were that the relative humidity fell to below 35%, and the locusts ate little and produced dry faecal pellets. Drinking of water droplets and chewing damp soil was observed, but evidently this was not enough to replenish water reserves, as dissected locusts appeared desiccated, although fat was plentiful. Albrecht also observed that females were more resistant to desiccation (cf. page 18). Gangwere (1960) reported qualitative field observations on drinking in Michigan Orthoptera, concluding that it was an infrequent occurrence.

The uptake of liquid water in insects through the general body surface or through specialized regions other than the mouth and anus has received little attention except in the egg stage which has been much studied, particularly in Orthoptera. It has been claimed by Colosi (1933) that *Acridium* (*Anacridium*) *aegyptium* adults were able to absorb water through the integument, and this is the only case on record for an acridid. Buxton (1930) showed that the mealworm *Tenebrio molitor* gained as much as 18% in weight when starved at 90% R.H., 30°C and 23°C, but attributed this to retention of metabolic water. Buxton later (1932a) revised this opinion, and his hypothesis that absorption of water vapour would account for the phenomenon was confirmed by Mellanby (1932), who showed that uptake occurred irregularly in mealworms above 88% R.H. – a fact which has since been confirmed many times. Bodine (1921) claimed that hibernating *Chortophaga viridifasciata* nymphs took up water in saturated air, but the possibility of other means of uptake was not adequately excluded. Ludwig (1937), however, has amplified Bodine's findings and shown that absorption of water vapour in *Chortophaga* will occur in air at about 82% R.H. Most water was gained at 96% R.H., and there were gradual losses after 20 hours of exposure. Although the phenomenon of water vapour absorption occurs in some insects (Edney 1957), it has been shown *not* to occur in *Periplaneta* (Edney 1966), *Rhodnius* (Buxton 1932b) and *Glossina morsitans* adults (Jack 1939).

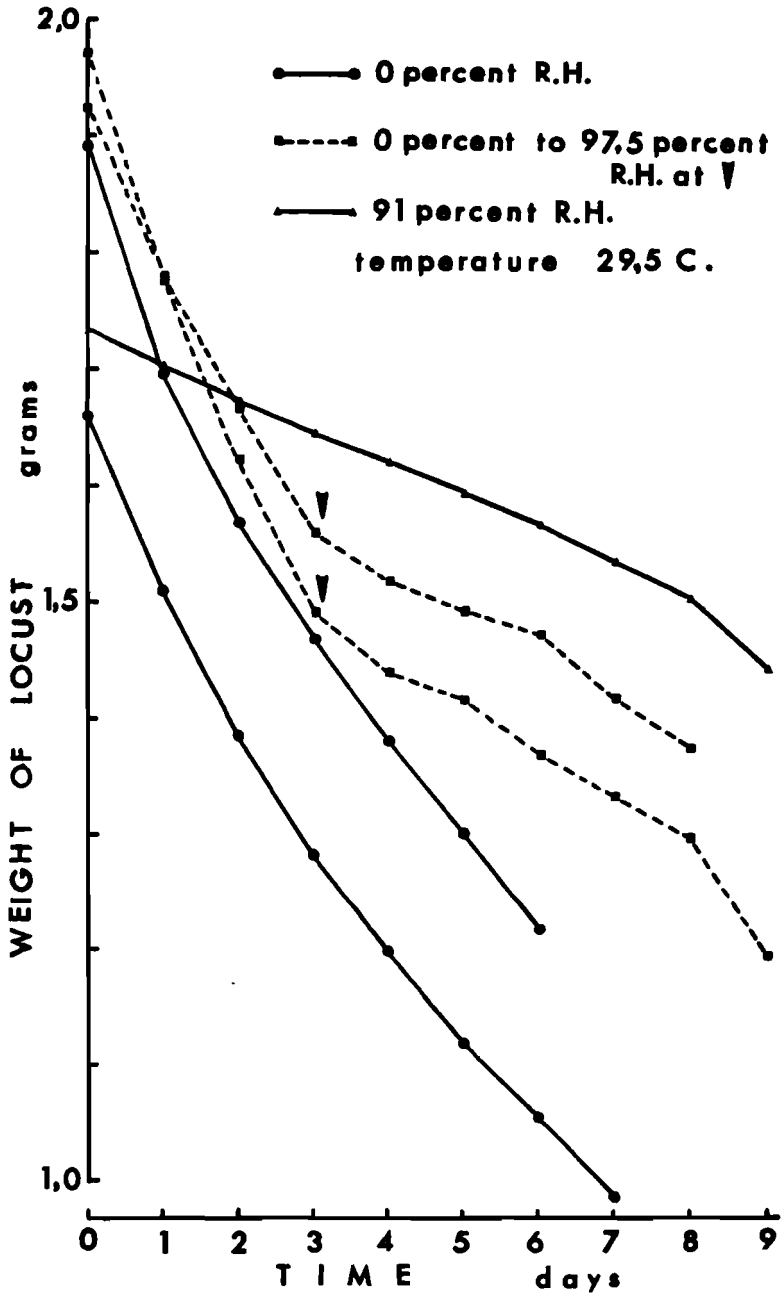

FIGURE 1
Change in weight of *Locusta* starved to death at different humidities, 29,5°C. Note that when transferred from 0% R.H. to 97,5% R.H. they did not gain weight.

Locusta adults are unable to gain water by absorption of contact water or water vapour. It has been shown that the locust is able to drink, that the amount of water drunk is correlated to some extent with water requirements, and that quite large amounts (up to 120 mg/g weight of locust) can be taken in a short while. The three methods by which locusts are able to gain water are:

(i) from metabolic water,
(ii) from the food, and
(iii) by drinking.

TRANSPIRATORY WATER LOSS

The contribution of cuticular water loss and water loss from the respiratory surfaces (spiracular) to total transpiration varies considerably amongst insects. Edney (1957) stated that spiracular losses contributed 60% of the total transpiratory loss in insects, but Bursell (1964) for example, found that loss from the integument contributed 75% of the total transpiratory loss in inactive *Glossina*, but that when the flies were active for 30% of the exposure time, 60% of water was lost from the spiracles. Humidity, temperature and air movement are also important in consideration of transpiration (Edney 1957; Beament 1961; Bursell 1964), and insects have been shown to have a remarkable degree of control over water losses, particularly *via* the spiracles.

Aspects of the control of transpiratory water loss in *Locusta* have been reported upon (Loveridge 1968a, b). The purpose of this section is to bring transpiratory loss into perspective and to report upon aspects of control of spiracular water loss noted during feeding experiments (Loveridge in press).

Transpiration through the cuticle

It has been found (Loveridge 1968a) that water loss through the cuticle of adult *Locusta* was proportional to size and in a "standard locust" of 1,6 g weight, there was water loss of 5,63 $\pm$ 0,67 mg/locust/h through the cuticle at 30°C, 0% R.H. It was also shown that a straightforward rectilinear relation between water loss and humidity did not exist, the relationship measured with dead locusts with blocked spiracles being curvilinear, falling away at high saturation deficits. It was shown that this was due to a permeability change which could occur in locusts up to 10 h after death. Winston & Beament (1969) state that the level of activity of water in the pronotal cuticle of 5th instar nymphs of *Locusta* is maintained at a level below that of the blood. This might account for the low apparent permeability of the cuticle of adult *Locusta* if the phenomenon occurred in adults and if it were shown that the activity level is maintained after the animals were killed by exposure to cyanide, but Winston & Beament (1969) showed that the activity level in the cuticle of *Locusta* nymphs is not maintained for even as long as two hours after death. There appears to be no necessity in all cases to link restriction of transpiration at low relative humidities with absorption of water vapour at high relative humidities (Noble-Nesbitt 1969).

Assuming that water is lost mainly through the head and body cuticle (as opposed to legs and wings), a permeability value of 0,71 mg/cm²/h in dry air at 30°C can be derived from surface area–weight relationships (Loveridge 1968a). Makings (1968) has shown that the permeability

of the cuticle of Slifer's patches in *Schistocerca* is at least several orders of magnitude greater than this, and as much as 169 ± 52 mg/cm²/h at the rather high temperature of 45°C. This may account, in part, for extremely high variability in water loss of isolated portions of abdominal exuviae of *Locusta* (Loveridge 1967). Moreover, the permeability transition effect at 40°C and the siting of the main patches on the abdominal tergites beneath the wings may account for the rather gradual transition temperatures measured for the cuticle permeability of acridoids (whole animals) (Chefurka & Pepper 1955; Loveridge 1968a). Slifer's patches occur, too, on *Locusta* (Slifer 1951) and on most Acrididae examined (Uvarov 1966), and it is quite evident that the cuticular water relations of locusts are considerably complicated by the existence of a mosaic of permeabilities and mechanisms. In spite of these complications a permeability estimate of 0,022 mg/cm²/mm Hg/h has been given (Loveridge 1968a) which is well within the range of values for adult insects given by Bursell (1964).

Water loss through the spiracles

Loveridge (1968b) has shown that the rate of water loss through the spiracles is subject to a considerable degree of control, and that there is a strong correlation between spiracular water loss and the rate of abdominal ventilation. Observations on the abdominal ventilatory rate were made during feeding experiments (Loveridge in press) by timing 50 ventilatory movements with a stopwatch. These data provide additional evidence for the control of spiracular water loss and are given below.

(i) *Effect of water availability in the food on the ventilatory rate of* Locusta. Groups of fledgelings of both sexes were isolated, group (a) being fed fresh grass of high water content and bran, group (b) being given dried grass and bran, and group (c) being given dry grass and bran but allowed drinking water (Loveridge in press). The results of measurements of the abdominal respiratory rate of the locusts between the third and eighth days of the treatment are given in Table 4. They show that high rates of ventilation prevailed in groups (a) and (c), and that the pattern of ventilation (Loveridge 1968b) was largely regular. In group (b), however, the rate was low and the pattern frequently (43,3%) discontinuous. This implies that low feeding rates in group (b) tended to reduce respiratory metabolism (but see page 3 and Loveridge & Bursell in press), thus reducing ventilatory rate. Low activity observed in group (b) would have enhanced this effect. It has moreover been shown (Loveridge 1968b) that reduction in ventilation accompanies desiccation. All three factors must combine to reduce the rate of ventilation with a resultant loss in spiracular water loss. Loveridge (1968b) showed that in dry air at 30°C *Locusta* ventilating at 20/min lost 3,2 mg/g/h and those ventilating at 34/min lost 5,3 mg/g/h. If interpolation between, and extrapolation above, these values is valid it can be calculated that group (a) would lose 7,1 mg/g/h, group (c) 6,5 mg/g/h, and group (b) 4,1 mg/g/h (Table 4). The total weight loss of locusts in treatment (b) was however only of the order of 50 mg/day (Loveridge in press) so that, for a 1 g locust, the calculated 4,1 mg/g/h is about double the expected loss. It is possible that further reduction in ventilatory rate may occur during the night or in the absence of the observer, whilst the increased discontinuities in ventilation patterns would tend to conserve more water.

TABLE 4

Abdominal ventilatory rates of *Locusta* maintained on three different feeding regimes

Treatment	*Abdominal strokes/min*		
	(*a*) *fresh grass, bran*	(*b*) *dry grass, bran*	(*c*) *dry grass, bran, water*
	36,6 D	12,2 D	24,2 R
	31,9 R	14,9 R	96,8 R
	37,0 R	78,9 R	24,6 R
	28,8 R	11,7 D	20,3 R
	45,5 R	17,6 R	22,9 R
	34,9 R	26,1 R	60,0 R
	34,1 R	15,5 D	68,2 R
	37,5 R	12,9 R	78,9 R
	46,2 R	76,9 R	37,5 R
	32,6 R	19,5 D	32,6 R
	48,4 R	25,0 D	34,9 D
	34,1 R	21,1 D	22,1 D
	88,2 R	24,6 D	93,8 R
	39,5 R	30,0 R	24,4 R
	28,8 R	22,7 R	37,0 R
	42,9 R	36,6 R	65,2 R
	49,2 R	25,9 D	18,8 D
	42,9 R	17,9 D	25,2 R
	35,3 R	8,9 D	19,9 R
	44,8 R	27,3 R	29,4 R
	90,9 R	20,7 D	57,7 R
	41,7 R	39,0 R	51,7 R
	120,0 R	25,9 R	30,0 R
	28,8 R	30,6 R	25,9 R
	36,1 R	27,3 R	27,3 R
	30,6 R	18,8 D	103,4 R
	111,1 R	19,0 R	37,5 R
	40,5 R	29,4 R	51,7 R
		20,0 R	
		24,2 D	
Mean ($\pm$ 2×S.E.)	47,1 $\pm$ 9,2	26,0 $\pm$ 5,8	43,6 $\pm$ 9,5

D = Discontinuous ventilatory movements
R = Regular ventilatory movements

(ii) *Effect of water availability in the food on the ventilatory rate of* Chortoicetes. The methods and treatments were similar to those described above for *Locusta* (Loveridge in press). The results are given in Table 5. Ventilatory rates were higher than in *Locusta* in general, in spite of the temperatures being similar, but the same pattern was seen with a reduction in abdominal ventilation in the dry-food treatment (b). Associated with this reduction was a marked increase in the incidence of discontinuous ventilation. It may be assumed that the control of spiracular water loss in *Chortoicetes* could be similar to that in *Locusta*, but water balance problems might be different because *Chortoicetes* is a much smaller insect.

TABLE 5

Effect of feeding treatment on abdominal ventilation in *Chortoicetes*

Group	*Feeding treatment*	*Mean rate of ventilation (movements/min.)*	*Incidence of discontinuous patterns %*	*Number of measurements*
a	fresh grass, bran	59,9 ± 7,0	12,1	33
b	dry grass, bran	33,7 ± 6,3	45,8	24
c	dry grass, bran, water	58,4 ± 10,2	24,0	25

(iii) *Effect of water availability in the food on the ventilatory rate of* Schistocerca. Methods and treatments were similar to those given above for *Locusta* (but see Loveridge in press). In this case the results were complete enough to allow a graphical representation (Figure 2). Symbols with an adjacent "d" indicate that the majority of the readings used in calculating the mean showed a discontinuous pattern of ventilation. This phenomenon was confined to treatment (b) from the third day onwards, when feeding reduction became pronounced (Loveridge in press). Associated with discontinuous ventilation was a reduction in rate, and the means for treatment (b) were held below 20/min from the seventh day. High values of ventilatory rate were recorded from treatment (a), with a lower ventilatory rate in the case of treatment (c). This suggests that control of water loss by reduction in ventilation may be expected to occur in *Schistocerca* as well as in *Locusta*.

Total transpiration in dry air at 30°C is about 10,3 mg/standard locust/h, of which about 5,5 is lost through the cuticle and 4,8 through the spiracles (Loveridge 1968a, b). Similarly the figures are 9,1 mg/standard locust/h, 4,2 and 4,9 at 50% R.H., 30°C. The figures for spiracular water loss in particular are considerably reduced if the locust's water reserves are lowered, but it can be seen that 53,4% of transpiratory water loss is through the cuticle in dry air and only 46,2% in air

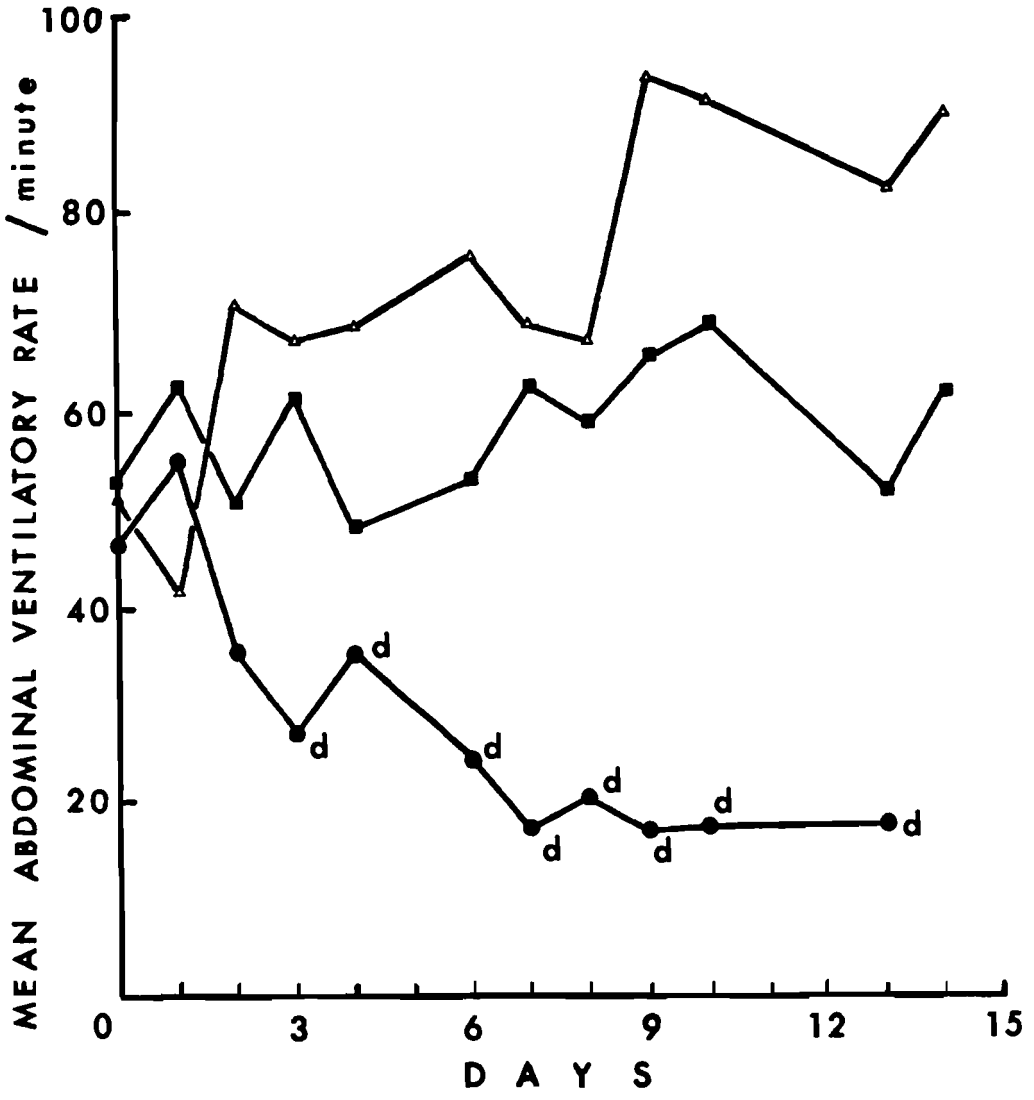

FIGURE 2

Rates of abdominal ventilation of adult *Schistocerca* fledging at 0 days with different feeding treatments. Triangles: group (a) fed on fresh grass and bran; circles: group (b) fed on dry grass and bran; squares: group (c) fed on dry grass, bran and given drinking water. The 'd' represents discontinuous ventilation.

at 50% R.H. Locomotor (walking) activity within the limits possible in a small box does not affect spiracular water loss (Loveridge 1968b). Further evidence for the control of water loss by reduction in ventilatory rate is given for *Locusta*, and similar evidence is presented for *Chortoicetes* and *Schistocerca*.

SURVIVAL IN RELATION TO FOOD AND WATER RESERVES

In a study of water balance of an insect it is pertinent to ascertain survival time under different environmental conditions. This gives a useful measure, for comparative purposes, of desiccation resistance. It is often impossible to know how much of the total water and metabolites (as measured) are available for utilization. It can be assumed that water and food reserves available to the insect are the amounts of those substances lost or consumed before death supervenes (Bursell 1964), but it is difficult to determine whether death is due to starvation or desiccation or both, or to some other factor or factors unknown. The water reserves of the insect have to be expressed in units independent of size if meaningful comparisons are to be made. In studies on the physiology of unfed *Glossina* the fat-free dry weight (R.D.W.), used as the unit of size (Bursell 1964), is a useful measurement, as R.D.W. is not subject to large fluctuations in different physiological states. In the locust, however, R.D.W. varies with age (Loveridge 1973) and with nutritional status (Loveridge in press). This is not to say that live weight (the unit of size used in this study) is subject to smaller fluctuations. However it does provide a more convenient size unit for comparative studies.

The water content of well-fed, mature adult locusts is between 62 and 74% of live weight (Loveridge 1973). In general, females have a slightly higher water content than males, but the range of variation is quite wide. Strong (1967) has shown that the increase in absolute weight of water during the adult life of female *Locusta* was associated with sexual maturation, and did not occur to the same degree in females reared without males. In the present work, females were always reared in the presence of males, and may therefore be regarded as "normal" with respect to rates of sexual maturation.

The fat content of mature adult locusts varies between 10% and 3% of live weight, and thus is considerably more variable than water content, but it shows a well-defined pattern of build-up and disappearance with the peak of 15–20 days after fledging (Loveridge 1973). Once again there is a sex difference, females having slightly less fat (% of live weight) than males.

The distribution of body water in Locusta

The distribution of water in blood, gut and the rest of the body may give an indication of how much water is indispensable to the locust, and so give an estimate of water reserves. Mellanby (1939) considered that one of the functions of the blood is to act as a store of water, and this view is reinforced by observations made on the amount of haemolymph in locusts fed on dry food (Loveridge in press).

To find the distribution of water in locusts aged 15–40 days, the blood volume was estimated by exsanguination (Sternburg & Corrigan 1959). It was observed that the blood from females was a clear green, while males had yellow-coloured blood (Uvarov 1966). The locusts were killed,

weighed and the gut removed and weighed. Gut and body were then dried and reweighed to determine the amounts of water contained in these tissues. Table 6 gives the results from 29 females and 17 male mature locusts. Of 1,4 g water (representing 69% of live weight), 20% was in the blood, 13% in the gut contents and tissues, and 67% in the rest of the body (Table 6, cf. Lee 1961). Although it is likely that some of the gut and body water could be lost without the locust succumbing, the weight of blood (280 mg) could be taken as a tentative estimate of water reserves in this sample.

Since the breakdown of food reserves yields roughly equivalent amounts of water, these must be considered in any estimate of water reserves. Depot fat constitutes 80% of the stored food of *Schistocerca* (Weis-Fogh 1952), and in this connection it will be regarded as being the sole food reserve of *Locusta*. The fat, carbohydrate and protein content of the fat body of adult female *Schistocerca* are given by Hill, Luntz & Steele (1968), and fats predominate throughout the first two gonotrophic cycles, but the other metabolites contribute a significant amount to reserves, and ignoring them will introduce an error which should be taken into account in the interpretation of results. Weis-Fogh (1952) found that a standard *Schistocerca* contained 10,3% (of live weight) lipid, of which 8,4% (175 mg) was in the fat body, 1,2% was indispensable lipid, 0,4% was in the legs and 0,3% in the wings. He found that locusts containing 2% or less lipid would not fly, and concluded that this fat was not available for metabolism. This figure may be somewhat higher in *Locusta*, since it has been found that this species died when extractable fat is reduced to 5,7% (Loveridge in press).

TABLE 6

The distribution of body water in mature *Locusta* (29 ♀♀, 17 ♂♂). Limits indicate ± 2 × S.E.

Total weight of water (g)	1,41 ± 0,13
Water (% live weight)	69,1 ± 1,4
Blood (% of total water)	20,2 ± 1,9
Gut water (% total)	12,8 ± 1,4
Body water (% total)	66,8 ± 2,5

The blood volume of Locusta

The weight of blood centrifuged from 22 male and 34 female *Locusta* was measured and the results are summarized in Table 7.

As the blood of insects contains appreciable concentrations of solids – for example the blood of *Arenivaga* (Dictyoptera) contains 16% solids (Edney 1966) – these weights are not good estimates of water reserves. Solids in blood of adult *Locusta* males and females were measured and found to increase with age, being 7,2% at fledging, 9,2% at 10 days and 12,4% at 20 days. Although the proportion of blood extracted by the exanguination technique is unknown, the weight of blood is similar to the volumes reported by Lee (1961) for *Schistocerca* using a dye dilution technique and by Loughton & Tobe (1969) for *Locusta*, measured by ^{14}C carboxyl-inulin dilution.

TABLE 7

Blood volumes of mature male and female locusts. Fiducial limits indicate $\pm$ 2 $\times$ S.E.

	Age (*days*)	*Live weight* (*g*)	*Blood volume* (*mg*)
Males (22)	17 $\pm$ 2	1,42 $\pm$ 0,08	157,3 $\pm$ 19,7
Females (34)	19 $\pm$ 1	2,30 $\pm$ 0,17	370,8 $\pm$ 50,2

The fat consumption of starving Locusta

In order to obtain an estimate of fat consumption independent of those reported by Loveridge & Bursell (in press), from oxygen consumption measurements, a sample of 44 male locusts about 50 days old was selected. Eighteen were killed and analyzed for fat content, while the remaining 26 were starved for 24 hours at 96% R.H., and then killed and analyzed. It was found that the control group contained an average of 5,07% fat, whilst the starved locusts contained 4,03% fat, showing a drop of about 1% in 24 hours. For a locust weighing 1,6 g this is 80 mg fat, reduced to 64 mg, a drop of 16 mg in 24 hours, or 0,67 mg/locust/h. In a locust weighing 1 g it amounts to 0,42 mg/g/h. This is high in comparison with the 0,28 mg/g/h calculated from figures of oxygen consumption (Loveridge & Bursell in press), and very high in relation to the figures of Gueutal (1941), and could perhaps be due to failure to allow for activity during the experiment.

Starvation and desiccation in Locusta

Millot & Fontaine (1937) claimed that adult *Schistocerca* fed on dry food, and then starved and desiccated, could tolerate reduction in water content to about 36,5% before death supervened. If this is so, it must represent a record for non-embryonic, non-diapause stages in insects, surpassed only by that of *Polypedilum vanderplanki* (Hinton 1960), since Millot & Fontaine claimed that the normal water content of their material was 71%. In experiments described elsewhere (Loveridge in press) I have been unable to repeat this work, finding that fledgelings fed on dry food until death, had a water content at death of 61,0 ± 6,1%. It is possible that differences would arise if the locusts were able to feed on fresh food until of normal mature weight and fat content, and then fed on dry food, but it is doubtful if, even then, they would survive until the water content was as low as 36,5%. Bursell (1964) states that the critical (lower lethal) water content in most insects is about 60%.

In an experiment to determine the critical level to which water can be reduced before death in *Locusta*, 30 adults (15 ♂♂, 15 ♀♀) 20 days old were isolated, and divided into three groups, each containing five males and five females. The control group was killed and analyzed for water and fat content, the remainder being starved in 100 × 40 mm gauze-topped tubes in desiccators at 29,5 ± 0,5°C in which humidity was controlled (Winston & Bates 1960). Locusts were considered dead when they no longer supported themselves above the bottom of the tube, and there were no responses to handling apart from twitching of antennae, tarsi and mouthparts. The second group was starved at 91% R.H. and the third starved at 0% R.H. These two groups were observed at frequent intervals and weighed daily, and dying locusts analyzed for water and fat content. The results are summarized in Table 8. The locusts starved at high R.H. lived for an average of 189 hours and lost 0,56 g in weight, while the locusts starved at low R.H. lived 134 hours and lost 0,80 g in weight. In both cases females appeared to be more resistant (Albrecht 1953), which is in accord with their larger water reserves but at variance with their lower fat reserves. However, fat body glycogen and protein (Hill *et al.* 1968) as well as reserves already deposited in the oocytes might have been available for metabolism by females. The control group had 1,34 g (61,6%) water and 220,5 mg (10,1%) fat, while locusts starved to death at 91% R.H. had 1,08 g (62,7%) water and 109,8 mg (6,6%) fat. The cause of death in this case was apparently starvation, for the water content was normal. Although a fat content of 109,8 mg appears adequate to sustain life for a longer time, the reduction in R.D.W. from 0,62 to 0,53 g may indicate that other metabolites had been used. The group starved at 0% R.H. apparently died of desiccation, their water content being reduced to 0,84 g (55,1%) while their fat reserves stood at 137,8 mg (9,1%). This compares with earlier results (Loveridge in press) where locusts fed on dry food died when their water content was 56,7% and fat content 5,7%. It seems likely that the lethal lower limit of water content in *Locusta* is about 55% (on a fresh weight basis) and this level is reached before food reserves are exhausted, but the possibility of the exhaustion of specific metabolites contributing to death cannot be excluded.

On the basis of transpiration figures available for *Locusta* (Loveridge 1968b; see also page 13) it can be calculated that at 30°C and 0% R.H. the blood of female locusts would become exhausted in 25 hours, and that of males in 17 hours. This indicates clearly that blood cannot be the whole water reserve and that water loss does not continue at these initial rates, but that ventilatory control must reduce water loss, while reduction in R.D.W. enables more water to be

TABLE 8

Survival time, weights, water and fat contents of groups of 10 adult *Locusta* (5 ♂♂, 5 ♀♀) starved to death at high and low humidities.

Treatment	*Survival* (*h*)	*Initial weight* (*g*)	*Weight at death* (*g*)	*Dry weight* (*g*)	*Water content* (*g*)	*Water content* %	*Dry weight* (*g*)	*Fat content* (*mg*)	*Fat content* % *live weight*
Control – direct from cages	—	—	2,18	0,84	1,34	61,6±1,7	0,62	220,5	10,1±1,5
Starved at 91% R.H., 29,5°C	189±48	2,27	1,71	0,64	1,08	62,7±2,5	0,53	109,8	6,6±1,7
Starved at 0% R.H., 29,5°C	134±22	2,31	1,51	0,68	0,84	55,1±2,3	0,54	137,8	9,1±1,9

lost before the critical level is reached. While blood volume may give some indication of the state of the locust's water reserves, it cannot represent the actual amount of water the locust can lose before death occurs.

Fat consumption of locusts starved for long periods is not in agreement with the figures found over a short period (see page 17). From Table 8, locusts starved at 91% R.H., 29,5°C, consumed 111 mg fat in 189 hours, which is 0,59 mg/h for a locust weighing 2,3 g, or 0,26 mg/g/h. Locusts starved at 0% R.H. consumed 83 mg fat in 134 hours, which is 0,62 mg/h, or 0,27 mg/g/h for a locust weighing 2,3 g. The mean of these two values (0,2553 mg/g/h and 0,2693 mg/g/h) is 0,26 mg/g/h, and is in good agreement with fat consumption figures of 0,28 mg/g/h obtained from oxygen consumption data (Loveridge & Bursell in press). These data are more in line with the results of Gueutal (1941), who found that *Schistocerca* starved for 14 days in dry air lost 3% of fat.

Water reserves can be recalculated on the basis of weight of water lost in dry air before death. Loss of water is 0,50 g (difference between control and 0% R.H. treatments) to which can be added 89 mg of metabolic water derived from the consumption of 83 mg fat, giving a total of about 0,6 g water reserves. Loveridge (1968b) found that transpiratory water losses were about 6,2 mg/g/h at 0% R.H., 30°C (see also page 17). Thus, a locust of 2,3 g weight would be able to survive for 41 hours before the 0,6 g water reserves were exhausted and death supervened. The discrepancy between observed longevity (134 hours) and calculated longevity must be explained by the fact that locusts starving in dry air control the rate of water loss by reduction in rate and depth of ventilatory movements, and also by the increased incidence of discontinuous respiratory patterns (see page 11).

The normal water content of grasshoppers and locusts is in the range 80% to 60% on a fresh weight basis (Uvarov 1966). This is corroborated in the present work for *Locusta migratoria* (Loveridge 1973), *Chortoicetes terminifera* and *Schistocerca gregaria* (Loveridge in press). Aziz (1961) working with 5th instar *Schistocerca* showed that both at high and low R.H. the locusts maintained a water content of 75–76% when starved. Blackith (1961) showed that hatchlings of *Locusta*, *Schistocerca* and *Nomadacris* when starved and desiccated died when their water content was reduced to 63% (normal level 74–84% for *Schistocerca*). Bodine (1921) showed that the water content of adult American grasshoppers was 67–74%, falling with age, and the lowest naturally occurring water content was 65% in hibernating nymphs of *Chortophaga viridifasciata*. This could be reduced to 60% by desiccation. Ludwig (1937), also working on *Chortophaga*, showed the lethal level of water content in starvation/desiccation experiments was 56,1–58,8%. These figures are in good agreement with the levels found for *Locusta* in this work.

It has been shown that blood volume may be important in water balance, as blood may serve as a water store, and exsanguination elicits a drinking response. Lee (1961) observed fluctuations in blood volume which accompanied moulting, maturation, and dietary variations. Similar data for *Locusta* are recorded by Loughton & Tobe (1969). Barton-Browne (1964) has stated that insects are not bound to the maintenance of a definite blood volume so that the blood acts as a water store. But the reduction of blood volume is not accompanied by a commensurate rise in osmotic pressure (Stobbart & Shaw 1964). That blood volume may be important in water balance has been suggested by Mellanby (1939), and Phillips (1964), for example, found quite wide variations in the blood-osmotic pressure of *Schistocerca* adults depending upon

treatment prior to blood sampling. Locusts fed hypertonic saline had blood of freezing point depression of 0,92°C, whilst locusts given tap-water to drink had a Δ°C of −0,72. Winston & Beament (1969) found that 5th instar *Locusta* starved for only 4–7 hours at various humidities showed changes in blood osmotic pressure. The relevant humidities and mean depression of freezing points of haemolymph were as follows: dry air −0,676°C; 42% R.H. − 0,662°C; 76% R.H. − 0,651°C and 85% R.H. − 0,656°C. The collection of blood samples from adult locusts is not possible when they are reared under dry conditions (Hoyle 1954; Phillips 1964), so this and the blood osmotic pressure data given above would suggest that the blood serves as a water store in locusts, as in other insects. Blackith (1961) has suggested that lysis of proteins to augment the osmotic pressure of the blood of starving hatchling locusts is a water conservation mechanism. Amino acids are perhaps utilized as respiratory substrates during starvation, or are mobilized for repair of damage when the cuticle has become abraded. Blackith's conclusions are not in accord with the findings of Djajakusumah & Miles (1966), working on *Chortoicetes*.

It is concluded that the water content of adult *Locusta* is normally 62–74% depending on age. The lethal lower level is 55%, but starvation during desiccation will result in dry weight losses which will eke out the water reserves. Blood volume is high at fledging, and falls to a constant level after about 10 days. The blood serves as the primary water store in the locust, and locusts reared in dry conditions or desiccated have little blood. Fat serves not only as a food reserve, but also as a water reserve, since for every milligram of fat consumed during respiration, slightly more than one milligram of water is produced. It would appear that between 2% and 5% (of body weight) is the non-usable lipid – held probably as cuticular waxes and lipoproteins.

WATER BALANCE

The ultimate aim of this work was to draw up a balance sheet of the water relations of the locust, and to determine the relative contributions of the various paths of water gain and loss under a few controlled conditions.

Locusts were starved for 24 hours at 96% R.H., and thereafter their water intake and faecal output when fed on fresh grass was measured (Loveridge in press). They were then starved for about 16 hours at 96% R.H., and total transpiratory water loss measured at 50% R.H., 30°C (Loveridge 1968a). They were then killed in HCN, and cuticular water loss at the same temperature and R.H. was measured, so with two readings of total transpiration and three readings (each over half an hour) of cuticular water loss, spiracular water loss could be calculated. All the separate contributions were calculated as mg/g/h, so they are not directly comparable to the figures in mg/locust/h calculated from values from other experiments shown in Figure 3.

The results of water balance measurements from nine experimental animals are given in Table 9. Fed locusts produce 0,4 mg/g/h metabolic water (Loveridge & Bursell in press) so this figure was entered in the table, although not actually measured in these individuals. The results show that water was gained at 42,9 mg/g/h, and lost at 18,3 mg/g/h. It would seem that the

apparent discrepancy can be attributed to abnormal patterns of feeding behaviour after starvation (Blaney & Chapman 1970) which give an overestimate of water intake with the food. The water balance of the locust is dynamic, continually changing in response to environmental and internal factors. The figure for cuticular water loss must be an over-estimate, since it is the mean of three values obtained during the time when cuticle permeability was high (Loveridge 1968a). If this figure is too high then the figure for spiracular water loss is too low.

The water balance of "standard locusts" is represented diagrammatically in Figure 3. Case A represents a locust fed on fresh grass (about 80% water), so that the intake of water was 51,3 mg/locust/h and loss 30,7 mg/locust/h (from Loveridge in press: locusts fed on grass take in 31,7 ± 5,5 mg/g/h and lose 13,5 ± 2,9 mg/g/h in the faeces). Metabolic water contributes 0,6 mg/locust/h to gain. As drinking is infrequent in locusts fed fresh grass, this is discounted. It can be seen, therefore, that gain was in excess of loss in this case (cf. Table 9).

When locusts are fed on dry food (about 5% water) – Figure 3B, the situation is very different. The intake of water in this case was 0,2 mg/locust/h and this was accompanied by loss of 1,6 mg/locust/h in the faeces. (Calculated from 0,14 ± 0,02 mg/g/h and 1,0 ± 0,3 mg/g/h: Loveridge in press). Metabolic water contributes 0,6 mg/locust/h to the gain, and as much as 192 mg/locust in half an hour could be gained by drinking. Since it is unlikely that drinking-water

TABLE 9

Water balance of locusts fed grass. Fresh grass (Kikuyu) = 80,0 ± 1,9% water. Water loss during exposure to 50% R.H., 30°C

		Water gain		*Water loss*		
Sex	*Age (days)*	*In food (mg/g/h)*	*Metabolic (mg/g/h)*	*Faecal (mg/g/h)*	*Cuticular (mg/g/h)**	*Spiracular (mg/g/h)†*
♂	17	61,2	0,4	7,6	3,5	0,4
♂	17	52,6	0,4	9,6	3,5	1,5
♀	17	32,0	0,4	7,8	2,6	1,1
♀	55	65,9	0,4	32,1	5,2	1,0
♀	59	36,3	0,4	13,7	6,0	1,2
♂	54	43,2	0,4	16,0	3,6	2,2
♀	18	27,5	0,4	7,5	2,3	0,9
♀	17	29,7	0,4	15,8	2,8	1,6
♀	18	34,1	0,4	11,7	2,9	0,9
Mean ± 2 × S.E.		42,5 ± 9,5	0,4	13,5 ± 5,2	3,6 ± 0,8	1,2 ± 0,3

* Mean of 3 readings over 1½ hours
† Mean of 2 readings over 2 hours

would be available in nature under very dry conditions, this source can be omitted in total gain. It was found (Loveridge 1968b) that conservation of as much as 2,6 mg/g/h of spiracular water loss could be effected in desiccated locusts when exposed to 50% R.H., 30°C. Thus sources of loss are spiracular (0,8 to an estimated 2,3 mg/locust/h), cuticular (less than 4,2 mg/locust/h) and faecal (1,6 mg/locust/h). This gives a total gain of 0,8 mg/locust/h against a loss of 6,6 mg/locust/h.

The most important single factor involved in the water balance of *Locusta* is the gain of

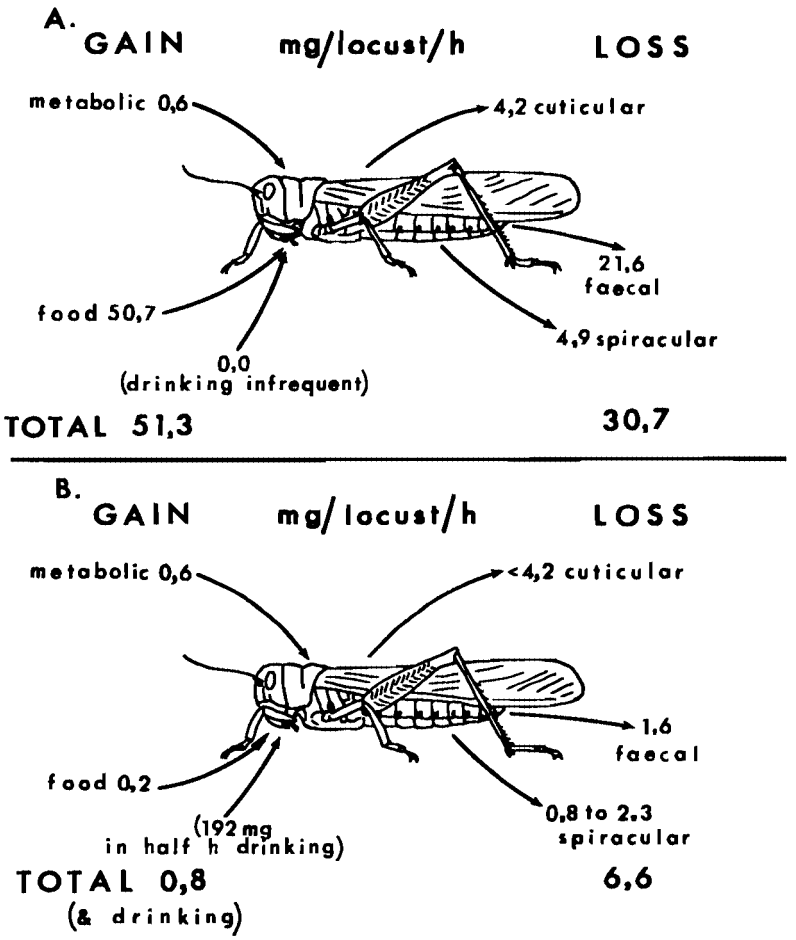

FIGURE 3

The water balance of 'standard' locusts (data reduced to 1,6 g size) at 50% R.H., 30°C. A. Locust feeding on fresh, green grass. B. Locust feeding on dry food. Components of water gain and total gain are listed on the left, and components of loss and total loss are listed on the right in each case.

water with the food and loss in the faeces. Hill, Luntz & Steele (1968) showed the water intake of *Schistocerca* fed fresh food to be 1 500–2000 $\mu\ell$/day which agrees well with 1 230 $\mu\ell$/day which can be calculated from the figure for water intake given above, particularly if species size differences are considered. The most potent method of conservation of water reserves is the reduction in food intake when only dry food is available (Loveridge in press).

The regulation of water balance

With some of the factors affecting water balance in the locust discovered, it is pertinent to discuss the possible internal mechanisms which regulate this balance. Humidity, temperature and the water content of the food have been shown to be the main external factors affecting water gain and loss in the insect. Perhaps the most powerful regulation mechanism available to the locust is behaviour (Barton-Browne 1964), and this has not been assessed in this work. The humidity behaviour of locusts has been described by Aziz (1957) and their feeding behaviour by Blaney & Chapman (1970). Evidently a preference for high R.H. or food of high water content (perhaps both part of the same reaction) will lead to conservation or replenishment of water reserves. Low humidities will result in decreased ventilation in starved locusts (Loveridge 1968b), and a similar effect as well as reduced motor activity and faecal production is observed when locusts are fed on dry food (see page 11 and Loveridge in press).

There is mounting evidence for both diuretic and anti-diuretic hormonal effects on both the malpighian tubules and the rectum in insects (Wall 1967). As far as locusts are concerned, Chalaye (1966) found structures in *Locusta* similar to the neurohaemal organs described by Maddrell (1966) as being the sites of release of the diuretic hormone in *Rhodnius*, and Highnam, Hill & Gingell (1965) produced some evidence showing that the cerebral neurosecretory cells of *Schistocerca* produce a diuretic factor, but gave no direct evidence that the factor acts upon the malpighian tubules. Cazal & Girardie (1968) showed the presence of a diuretic factor from the pars intercerebralis of *Locusta* affecting the malpighian tubules in vitro, the same extracts having an anti-diuretic effect on the rectum. Cazal (1965) reported that removal or implantation of corpora cardiaca in adult male *Locusta* had no effect on water content, but Cazal & Girardie (1968) claim to have shown an anti-diuretic effect of extracts of corpora cardiaca on both malpighian tubules and rectum, an effect strongest in extracts from hydrated animals. Interesting work has been done on the hormonal control of water balance in both *Locusta* and *Schistocerca* (Mordue, *et al.* 1970; Mordue 1972), showing the existence of a diuretic hormone from the storage lobes of the corpora cardiaca acting upon both malpighian tubules and rectum and an anti-diuretic hormone acting upon the rectum alone. Distension of the foregut (as in feeding) has been shown by Bernays & Chapman (1972) to increase electrical resistance across the tips of the maxillary palps and to stimulate release of the diuretic hormone. They propose that foregut distension stimulates stretch receptors in the gut wall, thus causing release of hormone from the storage lobes of the corpora cardiaca. This hormone (which could be the diuretic factor) acts on the terminal sensilla of the palps, causing them to close, thus increasing the resistance. These facts help rationalize the very marked inhibition of feeding when *Locusta*, *Schistocerca* and *Chortoicetes* are offered dry food (Loveridge in press). Any production of diuretic hormone following distension of the foregut with dry food would result in excessive water loss in the faeces which could not be recouped from water in the food.

Delphin (1965) showed that the A_2 neurosecretory cells of the metathoracic and abdominal ganglia of *Schistocerca* (these ganglia are the centres of nervous control of respiration: Miller 1960), become active after flight or dehydration, indicating the possibility of some neurosecretory factor influencing reduction in rates of water loss. The experiments are, however, open to other interpretation including mobilization of food reserves during starvation or increased metabolic activity. Strong (1967) showed that there are connections between feeding, sexual maturation and the periods of diuresis associated with periods of high feeding activity in *Locusta* females and that these processes are under hormonal control.

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