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JEB CLASSICS

INSECT FLIGHT TAKES OFF



Raul Suarez and Jon Harrison write about August Krogh and Torkel Weis-Fogh's 1951 ground-breaking publication on insect flight physiology.

This 1951 JEB classic paper, written at the dawn of the Golden Age of biology by Viking physiologists August Krogh and Torkel Weis-Fogh, was a collaboration between two men at opposite ends of their careers (Krogh and Weis-Fogh, 1951). August Krogh, the only comparative respiratory physiologist to earn a Nobel Prize, mentored Weis-Fogh during the last years of his life, and died before the publication of this paper. Among biologists, Krogh is most famous for a concept that is widely applied even today (Editorial, *Nature Genetics* **34**, 345-346, 2003); that for a large number of problems, nature has provided an animal of choice on which it can be most easily studied. This was only Torkel Weis-Fogh's third paper in a series that have all become classics in the fields of flight physiology and biomechanics. Weis-Fogh (1949) had previously shown that it was possible to induce locusts to fly for long periods of time on a tether by stimulating sensilla on the head with a jet of air. The 1951 paper combined Weis-Fogh's tethered flight technique with August Krogh's expertise in respirometry to provide the first measures of metabolic rate and respiratory quotient in a flying locust. Weis-Fogh's research in Krogh's laboratory was made possible by a grant from London's Anti-Locust Research Center, demonstrating that then, as today,

potential practical benefit can fuel basic research.

In retrospect, what is perhaps most impressive about this paper is that the authors were able to collect data that have stood the test of time with gas analysis instrumentation that was 100–10,000 times less sensitive than what we have today. For those who have not had the pleasure of using the Haldane method of O₂ and CO₂ analysis, it is a tricky, toxic business, in which samples of air are trapped within a volumetric syringe behind mercury, and the O₂ and CO₂ gas fractions calculated from the changes in gas volumes after they are solubilized by ferricyanide and NaOH, respectively. Measuring the gas exchange of a flying insect is more challenging than for a vertebrate, as the many spiracles preclude the use of a respiratory mask. To overcome the low-sensitivity of their gas analysis method, the Vikings devised a respirometry chamber equipped with a home-built pump that recirculated air onto the tethered locust's head, providing the stimulus for flight. For the short time-interval studies of respiratory quotient in post-flight locusts, they allowed acidified water to flow in around the locust as they extracted the entire chamber volume, providing them with maximum sample from an animal maintained in the minimum possible volume. With these techniques they reported resting and flight metabolic rates very similar to those measured later with modern gas analyzers (Armstrong and Mordue, 1986; Greenlee and Harrison, 2004).

The composition of metabolic fuels is such that the oxidation of a given amount of carbohydrate produces the same amount of CO₂ as the O₂ consumed, while fat oxidation yields a ratio of CO₂ to O₂ of 0.7. Thus, the ratio of the steady-state rate of CO₂ production to O₂ consumption by animals, i.e. the respiratory quotient, can be used as an index of the nature of the fuel(s) used. Another important scientific finding reported in this paper was that, unlike all prior studies of insect flight, the respiratory quotient of flying locusts was less than 1, and tended to decline with time in flight. Up to the publication of this paper, respiratory quotient had only been measured for bees and fruitflies, which had been shown to utilize carbohydrates as the primary fuel for flight. This paper provided the first evidence (later confirmed, by Jutsum and Goldsworthy, 1976) that fat was the primary fuel in long-term locust flight. Since then, many investigators have used respirometric and biochemical methods to study temporal changes in fuel

use by insects and the diversity of fuel use among species. The fact that the fuel used to support flight seems to vary more with phylogenetic history than ecology/life history remains an understudied problem in this field.

Equally interesting is the, as yet, unresolved finding in this paper of elevated gas exchange rates for 1–2 h after flight. The study of ‘oxygen debt’ was popular when this paper was published, and new insights into this topic continue to emerge (Pinz and Portner, 2003). In the 1951 paper, Krogh and Weis-Fogh referred to this elevated post-exercise oxygen consumption as ‘oxygen debt’, but suggest that it is not due to lack of oxygen in the flight muscle. However, subsequent work has confirmed that flight metabolism is completely aerobic in sustained insect flight. This elevated post-flight gas exchange is unlikely to be due simply to passively declining thoracic temperature, which should occur a few minutes after landing. Are we observing the slow removal of neurohormonal factors (e.g. octopamine; Orchard et al., 1993) that elevate tissue metabolic rates, and perhaps spontaneous behavior? What portion of these represent the costs of resynthesizing the fuels catabolized in the previous flight? More than half a century later, these data could still provide grist for a grant application!

Studies of animal flight continue to address fundamental problems and remain one of the most vibrant areas of experimental biology. Biomechanists use high-speed video, physical models and advanced theory to understand how animals fly and steer (Sherman and Dickinson, 2004;

Usherwood and Ellington, 2002); this area now seems poised to provide the biological inspiration for engineers to design minute flying robots. Muscle physiologists are now able to go into insect thoraxes to directly measure stress, strain, frequency and oxygen consumption, allowing the estimation of power input, output and efficiency of active muscles (Josephson et al., 2001). Pathway flux rates and mitochondrial electron transfer rates in individual flying insects have been examined to yield insights into how insects achieve the highest metabolic rates known in the animal kingdom (Suarez et al., 2000). A whole arsenal of molecular techniques is now available to study the flight muscle contractile machinery as well as membrane ion channels and pumps (Vigoreaux, 2001). The evolution of insect flight itself is being examined (Marden and Thomas, 2003). The great experimental biologists, August Krogh and Torkel Weis-Fogh provided large shoulders on which many must stand well into the future.

A PDF file of the original paper can be accessed online:
<http://jeb.biologists.org/cgi/content/full/207/19/3251/DC1>

10.1242/jeb.01190

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THE RESPIRATORY EXCHANGE OF THE DESERT LOCUST
(*SCHISTOCERCA GREGARIA*) BEFORE, DURING
AND AFTER FLIGHT

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(With Four Text-figures)

During the last two decades the knowledge of the physiology of heavy muscular work has increased considerably, and the high metabolic rate of flying insects has attracted particular attention. Jongbloed & Wiersma (1935), Chadwick & Gilmour (1940), and Davis & Fraenkel (1940) have reviewed the literature concerning the metabolic rate of flying insects. More recent investigations dealt mainly with the nature of the fuels combusted or with other aspects of insect flight. Most of the experiments referred to below dealt with measurements of the oxygen consumption of insects performing flight movements in a relatively small container. In this respect and because generally the animals were suspended and the natural locomotion was prevented the conditions of flight were abnormal. However, these limitations concerning the conditions of flight are probably of minor importance when we are interested mainly in the order of magnitude of the metabolic rate and in the nature of the fuels combusted, and with these limitations in mind, the present state of knowledge might be summarized:

(1) During flight the oxygen consumption is considerably increased and has been estimated at about 100 l. O₂/kg. body weight/hr. in bees (Jongbloed & Wiersma, 1935), flies (Davis & Fraenkel, 1940), and butterflies (Zeuthen, in Krogh, 1941). This means that the metabolic rate during flight in some insects increases 100 times or even more compared with the metabolism during rest. In an extensive and very interesting series of experiments Chadwick & Gilmour (1940) and Chadwick (1947) have demonstrated that various species of *Drosophila* consumed only 20 l. O₂/kg./hr., a figure which was indirectly verified by Williams, Barnes & Sawyer (1943) and Wigglesworth (1949). In the above-mentioned papers by Chadwick, and a paper by Chadwick & Williams (1949), the metabolic rate was correlated with the wing-beat frequency.

(2) The respiratory quotient (R.Q.) during flight was measured in bees (Jongbloed & Wiersma, 1935, in *Apis*) and flies (Chadwick, 1947, in *Drosophila*). In both cases the R.Q. equalled unity, indicating the combustion of carbohydrates, and this interpretation seems to be correct, glucose and glycogen being utilized as fuels in *Apis* (Beutler, 1937) and *Drosophila* (Williams *et al.* 1943; Wigglesworth, 1949) respectively.

(3) After the cessation of flight a small oxygen debt was demonstrated in *Drosophila* (Chadwick & Gilmour, 1940). The debt was abolished in less than 2 min.

August Krogh's unpublished analyses of the R.Q. of flying Lepidoptera gave values about unity, and so it seems reasonable to assume that the higher insects derive the energy for flight movements from the combustion of carbohydrates. Many insects generally considered primitive from a morphological point of view are, nevertheless, excellent flyers in one or more respects. The dragonflies, for example, are second to none as far as the flying speed and the refined regulation of the flight movements are concerned, and locusts are able to endure sustained flight for several hours every day during a considerable period of time. As the migrations of locusts are of great economic importance, it is thus of interest from both practical and theoretical points of view to study the metabolism during flight in these relatively primitive insects. The purpose of the present paper has been to study some quantitative and qualitative aspects of the combustion of flying locusts.

MATERIAL

If not otherwise stated, the animals used for experiments were mature male *Schistocerca gregaria* (Forskål). They resembled the transiens phase in adult biometry and the gregarious phase in hopper coloration. They were bred in crowded cages at the Anti-Locust Research Centre, London, and were sent by plane from London to Copenhagen as young adults, usually less than 7 days after emergence, each individual being placed in a small plastic tube. The animals were undamaged and fresh on arrival. They were kept in cages at 27–30° C. and fed on green cabbage and dried green grass. The mortality was low and both sexes reached sexual maturity in 3–4 weeks after the final moult. A few days later oviposition began, indicating that the food was sufficient. Female locusts, under laboratory conditions, were disinclined to fly when they were loaded with eggs, and so only males were used. The experimental animals were generally taken directly from the cages, but in a few cases they were previously starved up to 72 hr., and in some cases it was tried to select the less active animals in order to facilitate the determination of the metabolic rate during rest.

METHODS

Measurements (to be described in a later paper) of the flying speed and the wing-beat frequency of *Schistocerca* flying in front of a wind tunnel revealed that variations in the temperature of the ambient air between 25 and 35° C. had only a slight effect upon the intensity of flight, in contrast to what Chadwick (1939) found in *Drosophila* in which the wing-beat frequency decreased considerably with decreasing temperature. For this reason we have only endeavoured to keep the temperature of the experimental room within 27–30° C. Some few experiments were performed at a lower (22° C.) or at a higher (35° C.) temperature.

All determinations of the oxygen consumption and the R.Q. were based on gas analyses performed by an analyser of the Haldane type, accurate to about 0.01%. An air sample of about 20 ml. was drawn from the container and was analysed for CO₂, O₂ and 'N', and the results were calculated according to Krogh (1920). Even when the initial composition of the air in the container might have been taken for

granted it was often, but not always, similarly analysed to correct for systematic variations in the analytical procedure. The double analyses represented in Table 2 (Exps. 1-7) indicate the reliability of the analytical method. The oxygen consumptions have been reduced to N.T.P.

For determinations during rest we adopted the following procedure. In the first series of experiments one or two animals were placed in a relatively large container of 100-200 ml. capacity filled at the desired temperature by a rapid stream of pure but moist atmospheric air. Generally the pressure was raised by 20-40 mm. of mercury above the atmosphere and read at intervals to make sure that the system was airtight. The animal or animals were kept enclosed for a suitable time in order to make the CO₂ content increase approximately to 1%. Control experiments in which the CO₂ percentage was raised artificially to 4-7% failed to show any significant increase of the metabolic rate in spite of the increased rate and depth of the respiratory movements. In other series of experiments on resting animals, we reduced the volume of the container to 20 ml. or less, ventilated by a rapid current of air, started the experiment suddenly by interrupting the ventilation, and connected the container to a gas-sampling vessel filled with mercury. This enabled us to do experiments of 5-15 min. duration which were brought to a stop by sucking practically all the air over into the gas-sampling vessel and replacing it with acidulated water. After such an experiment the animal had to be dried in an air current before it could be used again, but the procedure did not damage the animal in any way. This type of experiment was used especially to study the 'oxygen debt' incurred during flight.

For determination during tethered 'flight', we took advantage of the fact that a suitably suspended locust can be made to maintain flight movements for a long period of time if the aerodynamic sense organ on the upper part of the head is stimulated by a jet of air (Weis-Fogh, 1949, 1950). The locust was suspended from a small metal bar fixed to the prothorax with a sticky wax (10 g. beeswax + 4.5 g. colophony) and placed in the respiration chamber. In order to prevent the tarsi finding any support, the distal parts of the legs were sometimes cut off, and the wounds covered with wax, at least 24 hr. before the experiment. If the tarsi of a locust are able to contact the surrounding walls or even its own wings it stops immediately (cf. Fraenkel, 1932). The respiration chamber consisted of a container in which the air could be circulated by a pump and delivered as a jet directed towards the upper part of the head. The pump (Fig. 1, *A*) consisted of rubber tubing in which the air was driven forward by means of rollers (Krogh, 1904). In the preliminary series of experiments a glass container of 1180 ml. capacity was used, but when it became desirable to do determinations of a few minutes' duration only, we used a box of Plexiglas (Fig. 1) holding at first 517 ml. and later 532 ml., on account of an extension (*B*) partly made of wide rubber tubing distally closed by a rubber plug. This extension made it easier to adjust the air jet. However, CO₂ is slightly soluble in rubber and therefore it became necessary to determine the rate of the disappearance of CO₂ under the experimental conditions. At the CO₂ tensions in question the loss of CO₂ corresponded to 0.005-0.01% per experiment as far as the large and the 517 ml. containers were concerned and so it could be neglected (Table 2, Exps. 1-9),

the accuracy of the gas analysis being 0.02%. But in the 532 ml. container the rubber extension caused a loss corresponding to 0.02–0.05% per experiment, and the values in Table 2, Exps. 10 and 11, have been corrected accordingly.

When successive experiments were performed with the same animal, the pump and the locust continued to work, moist atmospheric air being blown through the container in order to renew the air during the intervals between the experiments (in at *C*, out at *D*). After 2–3 min. of ventilation, a sample of the air in the container was taken for analysis (at *E*) and a new experiment was then started by interrupting the air current. The temperature and the pressure (manometer connected at *F*) were

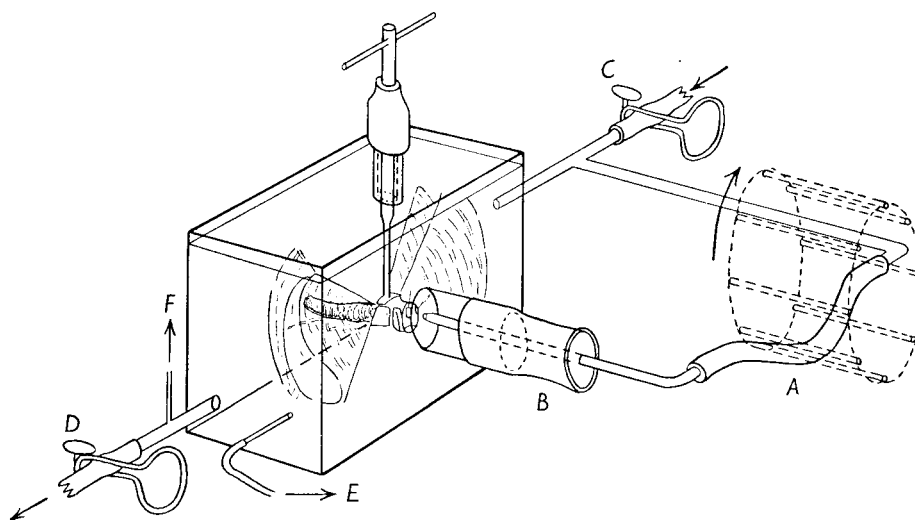


Fig. 1. Respiration chamber with a suspended *Schistocerca* which is stimulated by means of a jet of air to perform flight movements. For further explanation, see text.

controlled at the beginning and at the close of each experiment. During an experiment the percentage of CO_2 was allowed to increase from about 0.05 to about 1%. It was possible to make a locust fly continuously for $1\frac{1}{2}$ hr. when the air jet was properly adjusted.

RESULTS

Before flight

In order to get a rough estimate of the relationship between the metabolic rate in resting locusts and the temperature, a small series of experiments was performed in which mature males were enclosed in a fairly large container for 2–3 hr. at different temperatures. The results are plotted as circles in Fig. 2, in which the metabolic rates are given as litres of oxygen consumed per kg. body weight per hour. It is seen that the metabolic rate at about 30° C. could serve as a reasonable basis of comparison between the exchange during rest and flight since the intensity in resting animals varied roughly linearly with temperatures between 25 and 35° C., i.e. within the range of temperatures (no heat radiation) where sustained flight generally could be

performed in the laboratory. The R.Q. of the insects which were taken directly from the cage averaged 0.82 ± 0.03 .

In order to get a representative value for the respiratory exchange in resting animals a series of twenty-three experiments was performed at $27-30^{\circ}\text{C}$. Single mature males were taken from the cages and placed in a 20 ml. container for 6-18 min. Table 1 contains the results. As was to be expected, the metabolic rate varied to a considerable degree (Fig. 2, crosses), the average value amounting to 0.63 ± 0.03 l. $\text{O}_2/\text{kg./hr.}$ (s.d. 0.13) at 28°C . But this accuracy was sufficient for our purpose. The R.Q., on the other hand, was rather constant and averaged 0.82 ± 0.013 .

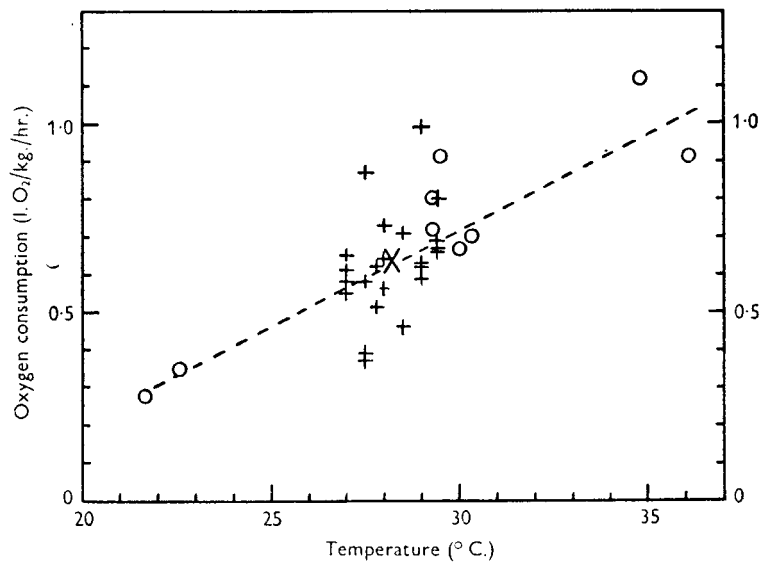


Fig. 2. Oxygen consumption of resting *Schistocerca* males in relation to temperature.

After the experiments the same locusts were made to fly for shorter or longer periods, and after the cessation of flight the respiratory exchange was followed (see later, Table 3).

During flight

Table 2 summarizes eleven experiments (twenty-five measurements) on the respiratory exchange of flying *Schistocerca* males. As in Table 1, the results are given partly as the oxygen uptake in cu.mm. oxygen consumed per animal per minute and partly as l. $\text{O}_2/\text{kg.}$ body weight/hr. Exps. 1-6 refer to the initial 12-30 min. of flight, whereas Exps. 7-11 show the results obtained during successive intervals of uninterrupted flight, the maximum flying time being 101 min.

Under these artificial conditions one could not expect to get any constant figure for the oxygen uptake during flight, and in fact the oxygen consumption varied between 10 and 30 l. $\text{O}_2/\text{kg./hr.}$ corresponding to 300-1000 cu.mm. $\text{O}_2/\text{male/min.}$ The average consumption was 15 l. $\text{O}_2/\text{kg./hr.}$ This means that the metabolic rate during flight had increased fifteen to fifty times compared with the value during rest.

Table 1. Respiratory exchange during rest at 27–30° C.

(Mature *Schistocerca* ♂♂, directly from the cages. Small containers.)

Date (1949)	Animal no.	Weight (g.)	Tempera- ture (° C.)	Duration of experi- ment (min.)	Respiratory exchange		
					O ₂ uptake per animal per min. (cu.mm.)	O ₂ uptake (l. O ₂ /kg./hr.)	R.Q.
15. ii.	III	1.9	27.5	6.42	28	0.87	0.83
15. ii.	VI	1.6	27.5	5.67	10	0.37	0.90
16. ii.	VI	1.6	27.8	11.40	16	0.62	0.84
21. ii.	VI	1.6	28.0	11.55	15	0.56	0.76
23. ii.	VI	1.5	29.4	11.67	17	0.69	0.76
23. ii.	VI	1.5	29.4	17.72	17	0.67	0.74
15. ii.	VII	1.5	27.5	5.25	10	0.39	0.90
16. ii.	VII	1.5	27.8	10.20	12	0.51	0.65
21. ii.	VII	1.5	28.0	10.45	18	0.73	0.84
21. ii.	VII	1.5	28.0	10.40	16	0.64	0.78
23. ii.	VII	1.6	29.4	13.47	21	0.80	0.85
23. ii.	VII	1.6	29.4	14.60	18	0.66	0.84
10. iii.	VII	1.6	27.0	17.43	17	0.65	0.80
10. iii.	VII	1.6	27.0	17.27	15	0.58	0.84
10. iii.	VII	1.6	27.0	17.35	16	0.61	0.79
10. iii.	VII	1.6	27.0	17.32	15	0.55	0.81
25. ii.	X	1.6	29.0	12.22	17	0.62	0.88
25. ii.	X	1.6	29.0	12.78	26	0.99	0.90
15. ii.	XVII	1.4	27.5	6.25	14	0.58	0.90
25. ii.	XVIII	1.5	29.0	12.18	16	0.63	0.83
1. iii.	XVIII	1.5	28.5	14.48	18	0.71	0.79
1. iii.	XVIII	1.5	28.5	15.43	12	0.46	0.74
25. ii.	XXIII	1.8	29.0	13.55	18	0.59	0.88

Oxygen consumption average 0.63 ± 0.03 l. O₂/kg./hr. S.D. = 0.13.
Respiratory quotient: average 0.82 ± 0.01 l. O₂/kg./hr. S.D. = 0.06.

Although these figures are of a moderate size compared with the figures for flies and bees, formerly referred to, they are high enough to show that the metabolism was radically altered, and presumably resembled the metabolism during natural flight both as far as rate of combustion and nature of the fuels are concerned. Consequently, a closer analysis of the variations of the R.Q. was of special interest. The experiments, nos. 7–11, revealed that the R.Q. at the end of the period of flight was always less than at the beginning, and if we average the figures obtained during the first 10–40 min. of flight in the experiments in which the animals were taken directly from the cages (twelve measurements) the R.Q. was 0.82 ± 0.01 , i.e. the R.Q. equalled the value before flight. If, however, we average the values obtained after at least 30 min. of flight we get 0.75 ± 0.02 . The difference between the two figures, 0.07 ± 0.02 , is statistically significant, and thus it has been demonstrated that the R.Q. decreased during sustained flight, indicating an increased combustion of fats. No correlation between metabolic rate and R.Q. could, however, be demonstrated.

After flight

It is possible to make a locust fly continuously for many hours under rather natural conditions if it is properly suspended in front of a wind tunnel or if it is attached to a round-about (Krogh & Weis-Fogh, 1951). After sustained flight, the

Table 2. *Respiratory exchange during flight*
(*Schistocerca* ♂♂.)

Exp. no.	Weight (g.)	Temperature (° C.)	Time after the beginning of flight (min.)	Respiratory exchange		R.Q.	Sexual development. Treatment before the experiment
				O ₂ uptake per animal per min. (cu.mm.)	O ₂ uptake (l. O ₂ /kg./hr.)		
1	—	34.5	0-11.5	780	—	0.82	} Mature. Directly from cage
				750	—	0.82	
2	1.9	29.9	0-29.0	600	18.8	0.79	} Mature. Directly from cage
				620	19.4	0.75	
3	2.0	22.9	0-30.5	490	14.8	0.74	} Mature. Has flown for 1 hr. about 3 hr. before the experiment
				490	14.9	0.74	
4	2.4	23.0	0-30.0	530	13.4	0.81	} Mature. Directly from cage
				540	13.8	0.81	
5	2.4	30.2	0-30.0	600	15.1	0.83	} Mature. Directly from cage
				620	15.6	0.81	
6	2.4	30.2	0-21.5	770	19.2	0.73	} Same individual as in Exp. 5 after 30 min. of rest
				790	19.9	0.71	
7	2.2	30.0	0-9.5	900	24.8	0.83	} Mature. Starved about 20 hr. R.Q. during rest
				910	25.1	0.83	
8	1.6	26.5	13-32	1030	28.4	0.77	} 0.74
				1040	28.6	0.77	
9	1.6	27.3	0-18	390	14.4	0.88	} Mature. Directly from cage
				370	13.9	0.85	
10	1.5	28.0	0-19	300	11.2	0.84	} Mature. Directly from cage
				280	10.2	0.84	
11	1.6	27.5	0-18	320	11.9	0.82	} Mature. Directly from cage
				320	11.9	0.82	
9	1.6	27.3	21-41	360	13.5	0.84	} Directly from cage. Same individual as in Exp. 8 4 days later
				370	13.7	0.77	
10	1.5	28.0	43-61	330	12.5	0.77	} Directly from cage. Same individual as in Exp. 8 4 days later
				340	12.7	0.73	
11	1.6	27.5	64-82	340	12.7	0.73	} Directly from cage
				340	12.7	0.78	
10	1.5	28.0	84-101	370	14.7	0.82	} Immature. Directly from cage
				250	9.9	0.82	
11	1.6	27.5	45-67	310	12.4	0.69	} Immature. Directly from cage
				310	12.4	0.69	
11	1.6	27.5	0-18	440	16.5	0.79	} Immature. Directly from cage
				340	12.9	0.84	
11	1.6	27.5	20-37	340	12.9	0.75	} Immature. Directly from cage
				340	12.9	0.75	
11	1.6	27.5	42-64	340	12.9	0.75	} Immature. Directly from cage
				380	14.1	0.69	

locusts generally settled quietly where they were placed in the cage, they did not eat unless they were placed directly on the food, and the rate of the respiratory movements was increased. In short, the animals appeared to be tired, although they were able to resume flight when suspended again. In order to study the metabolic rate during this phase of recovery, the same animals as referred to in Table 1 were forced to fly for shorter (10 min.) or longer periods (60-195 min.) by application of either of the methods mentioned above. Immediately after the interruption of flight each animal was placed in a 20 ml. container and the respiratory exchange was followed during successive intervals of time, preferably in the same individual, but in some experiments different animals were used. The results are seen in Table 3. The average values of the oxygen uptake and the R.Q. before flight have been taken from

Table 3. *Respiratory exchange after flight at 27-30° C.*(Mature *Schistocerca* ♂♂: the same individuals as in Table 1.)

Date (1949)	Animal no.	Respiratory exchange before flight (from Table 1)		Dura- tion of flight (min.)	Time after cessation of flight (min.)	Respiratory exchange after flight	
		O ₂ uptake (l. O ₂ /kg./hr.)	R.Q.			O ₂ uptake (l. O ₂ /kg./hr.)	R.Q.
21. ii.	VI	0.67	0.76	10	0.5-5.5	1.49	0.80
					11-18	0.95	0.82
					25-37	0.70	0.82
					55-66	0.71	0.81
					80-92	0.68	0.74
23. ii.	VII	0.75	0.84	10	1.5-7	1.92	0.72
					27-38	1.15	0.61
					54-66	0.79	0.74
1. iii.	XVIII	0.57	0.77	10	0.5-6	1.34	0.89
					10-19	0.71	0.76
					28-41	0.68	0.66
					52-68	0.49	0.67
15. ii.	VII III	0.39 0.87	0.90 0.83	60 60	0.5-6	1.27	0.84
					7-12	0.77	0.76
					40-49	0.45	0.73
23. ii.	VI	0.66	0.75	91	1-6	2.10	0.70
					11-18	1.28	0.66
					25-36	1.03	0.69
					48-59	1.00	0.62
					78-90	1.18	0.75
25. ii.	XVIII	0.67	0.85	99	1-6	1.54	0.76
					11-18	0.96	0.83
					23-34	1.02	0.76
					41-54	0.96	0.77
					84-96	0.75	0.71
					128-142	0.74	0.66
21. ii.	VII	0.73	0.81	103	0.5-6	1.93	0.71
					11-19	1.13	0.91
					28-40	0.91	0.73
					46-60	0.81	0.72
					76-90	0.85	0.68
15. ii.	XVII VI XVII	0.58 0.37 0.58	0.90 0.90 0.90	120 120 120	1-6	1.14	0.73
					7-13	1.23	0.73
					19-29	0.55	0.85
9. iii.	VII	0.62	0.81	195	0.5-6	1.50	0.73
					12-20	1.23	0.77
					29-45	1.05	0.76
					61-80	0.82	0.70
					101-121	0.77	0.73
					152-169	0.77	0.69
16. ii.	VII XI VI XXV	0.51 0.62	0.65 0.84		1-6	1.11	0.86
					8-15	0.86	0.79
					16-24	0.93	0.83
					25-36	0.70	0.78
16. ii.	X XXIII III XVIII				2-12	1.31	0.79
					26-36	0.85	0.70
					47-57	0.63	0.71
					67-77	0.63	0.71

Table 1, and if we compare these results with the results obtained after even a short period of flight, it is seen that the metabolic rate just after the flight had stopped was increased by two or three times, and furthermore that the recovery lasted at least

Table 4. *The oxygen uptake after flight: the 'oxygen debt'*

Duration of preceding period of flight (min.)	Animal no.	Oxygen uptake in l. O ₂ /kg./hr. at different times after cessation of flight (min.)					O ₂ uptake before flight (l. O ₂ /kg./hr.)
		3.5	10	20	30	60	
10	VI	1.49	0.94	0.78	0.70	0.70	0.67
10	VII	1.92	1.73	1.46	1.22	0.78	0.75
10	XVIII	1.34	0.92	0.68	0.68	0.48	0.59
	Average	1.58	1.20	0.97	0.87	0.65	0.67
91	VI	2.10	1.58	1.18	1.04	1.02	0.66
99	XVIII	1.54	1.07	0.96	1.02	0.91	0.67
103	VII	1.93	1.36	1.04	0.93	0.82	0.73
195	VII	1.50	1.33	1.18	1.09	0.89	0.62
	Average	1.77	1.34	1.09	1.00	0.91	0.67

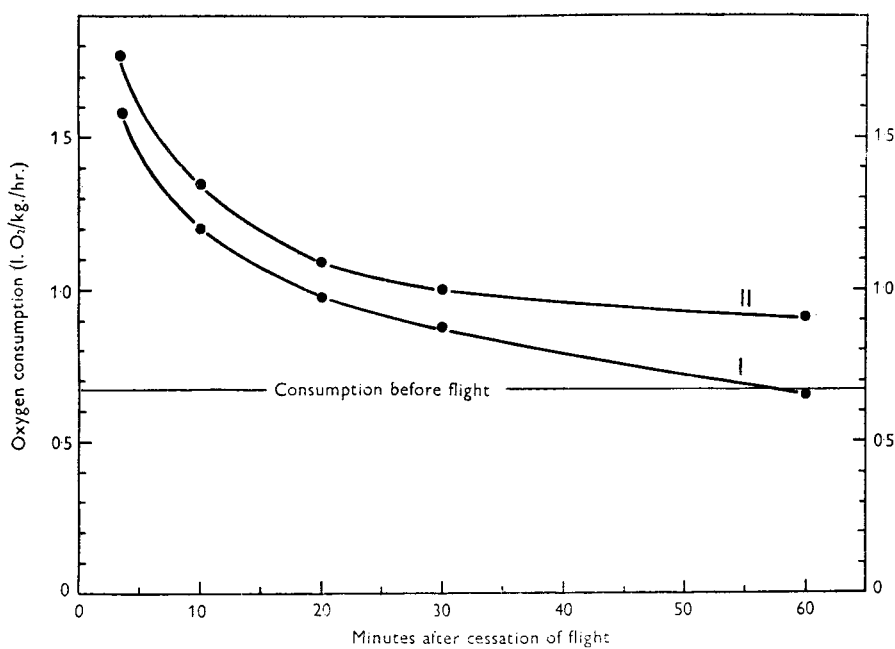


Fig. 3. Oxygen consumption and 'oxygen debt' of *Schistocerca* males after 10 (I) and 90-195 (II) min. of flight.

1 hr. In order to obtain a general expression of the increase in metabolic rate after flight, the oxygen consumption was plotted against time in the experiments in which the same animals were used during the entire period of recovery. By means of these

curves the rates could be read at arbitrary times, and Table 4 contains the figures read $3\frac{1}{2}$, 10, 20, 30 and 60 min. after the cessation of flight (seven experiments).

The average consumption after 10 (I) and 90–195 min. (II) of flight respectively are shown in Fig. 3. The horizontal line at 0.67 l. O₂/kg./hr. represents the average metabolic rate before flight of the seven animals in question, and the area between this line and the curves I and II represents the total increase in oxygen uptake after flights of short and long duration respectively. According to the general terminology this amount is referred to as the 'oxygen debt' incurred during flight. It corresponded to 0.3–0.7 l. O₂/kg., and it was very characteristic that the recovery lasted at least 1 hr. even after short flights, the total 'debt' as well as the time of recovery being doubled after prolonged flight. There was a general tendency of the R.Q. to decrease during the recovery, but certainly the R.Q. could not be relied upon until the metabolism had reached a steady state, i.e. at the end of the recovery. At this state the R.Q. was low (0.7) indicating a combination of fats. On account of possible anabolic processes, however, this interpretation is dubious.

ESTIMATION OF THE ACCURACY

As far as the oxygen consumption is concerned the capacity of storing oxygen in insects which do not contain haemoglobin is determined by the amount which can be physically dissolved, and as this amount is negligible, the accuracy of the measurements of the O₂ uptake was only limited by the analytical procedure. Generally the O₂ percentage of the air in the container was lowered by 1% and the CO₂ content increased correspondingly. This means that the oxygen consumed per animal could be determined with an accuracy of about 3%, the accuracy of the gas analyses corresponding to 0.02% O₂, while the estimate of the volumes of the small containers was accurate to 1%. But when the metabolic rate per kg. body weight was concerned, the figures were only correct to about 5–7%, the weight of the individuals varying 5% from day to day according to the content of the gut.

On account of the considerable amounts of CO₂ which are reversibly combined with the blood and tissues, it is difficult to estimate the sources of error involved in the determination of the R.Q. in flying insects, and furthermore only few investigations deal with the absorption of CO₂ in insect blood. In spite of this lack of appropriate data, the low R.Q., so unexpected beforehand, of flying *Schistocerca* justifies an attempt to estimate the effect of storing or washing out of CO₂. Christensen, Krogh & Lindhard (1934) and Christensen & Hansen (1939) discussed the requirements to be fulfilled in order to obtain reliable determinations of the R.Q., especially during muscular work of man, the main requirements with simultaneous determination of the CO₂ output and the O₂ uptake being: (1) the CO₂ expired during the experiment should by far exceed the amount of CO₂ which might be washed out or stored in the body, or if this is not the case, (2) the metabolism and the ventilation should have reached a steady state.

During the first minutes of flight the second requirement was certainly not fulfilled, and CO₂ might be washed out both due to a relatively increased ventilation, the raised body temperature, and the eventual decrease in the pH of the blood. No CO₂ dissociation curves of locusts are available and the only curves known to us refer to the blood of the honey-bee larva (Bishop, 1923) and the *Gastrophilus* larva (Levenbook, 1950). According to these curves the CO₂ capacity is about half of the capacity of human blood. The figures given by Florin (1937) indicated the same order of magnitude in some other insects. Liljestrand (1916) found that by maximum hyperventilation during rest 2–3 l. CO₂ could

be washed out of the human body, and during heavy muscular work Christensen & Hansen (1939) demonstrated a decrease in the CO₂ content of the blood amounting to 25 vol. %. This corresponds to 1 l. CO₂ washed out from the blood alone. According to the figures recorded by Liljestrand (1916) the maximum amount liberated from the body in excess of the amount produced by the combustion would then be 5 l. CO₂, the relation between the amount of CO₂ in the blood and in the remaining tissues being about 1:4. In other words, during muscular work in man the relatively increased ventilation, the increased body temperature, and the formation of lactic acid can cause 5 l. CO₂ to be washed out of the body. This figure corresponds to 70 ml. CO₂/kg. body weight. The minimum amount of CO₂ produced by flying *Schistocerca* was 8000 ml./kg./hr., and since no experiment lasted less than 10 min. the minimum amount produced during an experiment was 1300 ml. CO₂/kg. If we adopt the above figure from human beings, the amount of CO₂ expired in excess of the amount produced would not exceed $\frac{70 \times 100}{1300} \% = 5\%$ during the first 10 min. of flight. Since as a rule, the experiments lasted longer (one and a half to three times), and the metabolic rate was higher (one and a half to three times), the error would hardly exceed 2-3%. This accuracy was sufficient, the gas analysis allowing the R.Q. to be determined with an accuracy of 2% only. In *Gastrophilus* Levenbook (1950) found that, contrary to man, the larval tissues contained similar amounts of CO₂ per unit volume as did the blood. If this is the case in locusts, too, more than 70 ml. might be washed out or stored. However, the dissociation curves show that if the CO₂ percentage in the tracheal system was altered by 1.5% only 60-70 ml. CO₂/kg. would be deliberated or stored. In the above calculation, this means that the lower CO₂ capacity of insects would compensate the effect of the relatively higher CO₂ content of the tissues; and, in fact, the measurements of the R.Q. during the first period of flight did not differ significantly from the values obtained immediately after (Table 2, Exps. 7-11).

During the successive periods of flight the animals were to be considered in a steady state and thus the gas analysis alone determined the accuracy. The same holds true in resting animals when the large containers were used, the liberated CO₂ corresponding to 1000-1500 ml. CO₂/kg./experiment. When the small containers were used, however, analogous calculations gave a much lower accuracy, but since the average R.Q. before flight was 0.82 in both series, the animals were offered the same food, and the standard deviation was only 0.06 in twenty-three experiments with the small containers, it was concluded that the locusts were in a steady state during the experiments, and so it is reasonable to consider the values of the R.Q. before and during flight a valid expression of the proportion between the CO₂ produced and the O₂ simultaneously consumed, and thus an indicator of the materials combusted. But during the initial phases of recovery the rapid changes in metabolic rate and ventilation made the results less reliable.

DISCUSSION

Oxygen consumption and oxygen debt

The diagram, Fig. 4, illustrates the changes in oxygen consumption when a locust started to perform flight movements at a moderate intensity (15 l. O₂/kg./hr.) and flew for 10 min. only. Nevertheless, the diagram gives an impression of the enormous change in the metabolic rate at the transition from rest to work, and when it is remembered that the intensity during flight might be twice as big as shown in Fig. 4, and that flying might be continued for several hours, it becomes clear that the call for oxygen and nutrients are of quite another order of magnitude in a flying than in

a resting insect. The average increase in oxygen consumption was twenty-five times the value during rest, but sometimes the metabolic rate increased fifty times. The figures do not allow an estimate of the average consumption of migrating locusts. The wing-beat frequency of a locust flying in front of a wind tunnel under rather natural conditions only differed slightly from the frequency measured when the same locust flew in the container, and so it is reasonable to think that the metabolic rates of migrating locusts do not differ essentially from the rates measured in the container. In any case, every physiological mechanism of importance during flight, like the mechanisms of ventilation, combustion, fuel mobilization, heat regulation, and so on, must be demonstrated to work at a sufficient intensity at the highest metabolic rates measured so far.

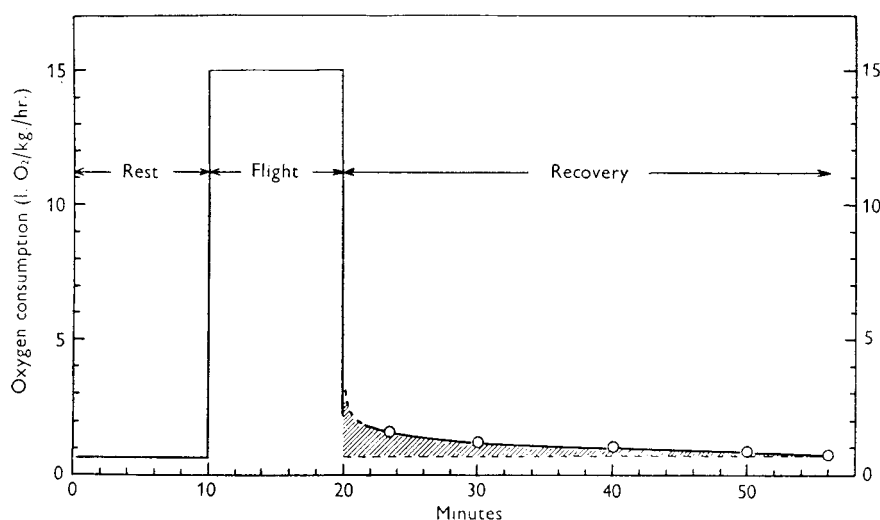


Fig. 4. Diagram. Oxygen consumption of *Schistocerca* males before, during, and after 10 min. of flight at moderate intensity. The hatched area represents the 'oxygen debt'.

The increase in metabolic rate after flight which characterized the phase of recovery amounted to 0.3–0.7 l. O₂/kg. in total. This 'oxygen debt' corresponded to the consumption during 0.5–1.5 min. of flight, only, and so anaerobic breakdown of nutrients during flight could not have amounted to much. The most conspicuous feature, however, was the considerable time, 1–2 hr., necessary to abolish the 'debt'. At the end of the recovery after even a short period of flight the R.Q. was always low (0.7), but this might be due to anabolic as well as to katabolic processes. In a later paper the supply of oxygen to the wing muscles will be dealt with: the ventilatory mechanism seemed to warrant that the 'oxygen debt' was due to other causes than lack in oxygen.

Comparison with other insects

An oxygen consumption of 10–30 l. O₂/kg./hr. in flying *Schistocerca* is considerable compared with the maximum uptake of 4 l. O₂/kg./hr. in man (Nielsen & Hansen, 1937), but the figures are of a moderate size compared with what has been found in

bees, flies and butterflies (cf. p. 344), and even in small mammals like mice and shrews metabolic rates of 20 l. O₂/kg./hr. have been recorded (Krogh, 1941; Pearson, 1947). However, the metabolism of flying insects has been studied most thoroughly in *Drosophila*, and therefore it is of special interest to compare the respiratory exchange of these small flies with the exchange of the much larger locusts. In spite of the great difference in body weight (1:1000) and the considerable differences in the structure of the wing apparatus, *D. repleta* consumes oxygen at a rate corresponding to 21 l. O₂/kg./hr. during tethered flight (Chadwick & Gilmour, 1940), i.e. at the same relative rate as a *Schistocerca* flying under similar conditions. But apart from the metabolic rate the metabolism during flight of these two types of insects differed considerably. Flying *Drosophila* exclusively utilized carbohydrates, whereas in flying *Schistocerca* the low values of the R.Q. showed that carbohydrates did not serve as the only source of energy in any of the experiments and, on the contrary, the lowering of the R.Q. as the flight continued indicated that the main source of energy during prolonged flight was fat. If we assume that the combustion of protein was negligible during flight compared with the combustion of fat and carbohydrates, the share of fats as a source of energy can be calculated. This assumption is reasonable, since in mammals the combustion of protein is practically independent of muscular work. The average R.Q. after more than 30 min. of flight was 0.75 only, which means that about 85% of the energy should derive from fats. It might be due to the difference in the fuels applied by the two insects that the 'oxygen debt' in *Schistocerca* was not abolished until 1-2 hr. after the flight had ceased, whereas the metabolic rate in *Drosophila* reached the resting level in less than 2 min. after the cessation of flight movements. Perhaps the elimination of ketone bodies resulting from the intensive breakdown of fats caused this prolonged recovery which was absent in *Drosophila*.

SUMMARY

The respiratory exchange of mature males of the Desert Locust *Schistocerca gregaria* (Forskål) has been studied during tethered flight in a small container and compared with the exchange before and after flights of varying duration. All determinations were based on gas analyses, and so the CO₂ output and the O₂ uptake were determined simultaneously. The accuracy of the analytical procedure has been discussed and the determinations of the R.Q. before and during flight found to be valid. The figures of the oxygen consumption are reduced to N.T.P.

1. During rest at 27-30°C. the oxygen consumption amounted to 0.63 l. O₂/kg./hr., which means that an average male of 1.8 g. consumed 18 cu.mm. O₂ per min. During flight, however, the consumption increased fifteen to fifty times, the corresponding figures being 10-30 l. O₂/kg./hr. or 300-900 cu.mm. O₂ per min. in an average male.

2. After even a short period (10 min.) of flight in a roundabout or in front of a wind tunnel a distinct 'oxygen debt' was demonstrated. After prolonged flight (90-195 min.) the 'debt' was nearly doubled. It corresponded to 0.3-0.7 l. O₂/kg. or to the oxygen consumption during only 0.5-1.5 min. of flight, so that anaerobic

processes could not amount to much. It was characteristic that the recovery lasted at least 1 hr.

3. The R.Q. in resting animals averaged 0.82, and during the first 30 min. of flight the same value was obtained, but during the following 60 min. a statistically significant decrease of the R.Q. could be demonstrated, the average value in this period being 0.75. This unexpected result strongly indicates that, unlike other insects investigated so far, locusts utilize mainly fat as a source of energy during sustained flight.

4. It is suggested that a surplus of ketone bodies caused by the intensive breakdown of fats might explain the prolonged recovery of 1–2 hr. duration in *Schistocerca*, the recovery after flight in *Drosophila* which fly on carbohydrate lasting only 2 min. or less in spite of the same relative rate of combustion.

This work is a part of an investigation on insect flight which was started by the late Prof. August Krogh, F.M.R.S. We are indebted to the Scandinavian Insulin Foundation (*Nordisk Insulinfond*) and to the Carlsberg Foundation (*Carlsbergfondet*) for financial support. We also thank Dr F. Buchthal who has undertaken the leadership of the laboratory after the death of August Krogh in September 1949. A grant from the Anti-Locust Research Centre, London, rendered possible the participation of the junior author, and special thanks are due to Dr B. P. Uvarov and to Dr D. L. Gunn for their help and interest. The Anti-Locust Research Centre delivered the locusts reared by Mr P. Hunter-Jones.

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