J. Exp. Biol. (1961), 38, 163–174
With 2 plates and 6 text-figures
Printed in Great Britain

FUEL UTILIZATION AND DURATION OF TETHERED FLIGHT IN *APHIS FABAE* SCOP.

BY A. J. COCKBAIN
Rothamsted Experimental Station, Harpenden, Herts

(*Received* 12 *August* 1960)

INTRODUCTION

The objects of this work were to determine the nature and amounts of fuel used during flight in *Aphis fabae* Scop., their histological distribution and their relation to the duration of tethered flight. The aphids were flown suspended in an air-stream of a speed equivalent to their own flight speed. How the metabolic rates of aphids flying in this manner compare with those during free flight is not known, but the quantitative results are comparable with those obtained for tethered flight of some other insect species (see Wigglesworth, 1949; Weis-Fogh, 1952; Hocking, 1953).

MATERIAL

Alate alienicolae and migrantes from laboratory culture or natural infestations were used.

Aphids in the laboratory were bred at $19 \pm 5°$ C. on broad beans (*Vicia faba* L., var. Claudia Aquadulce). For experiments, alienicolae from healthy colonies of the same clone were transferred to new plants $\frac{1}{4}$–$\frac{1}{2}$ hr. after the final ecdysis.

Aphids from natural infestations were from different plants; migrantes from spindle (*Euonymus europaeus* L.), alienicolae from curled dock (*Rumex crispus* L.), broad bean (*Vicia faba* L., Claudia Aquadulce) and winter bean (*Vicia faba* L., Garton's S.Q.). Parts of these plants with 4th-instar alate nymphs were cut off and stood in water in the laboratory; as ecdysis occurred the adults were transferred to small bean plants.

All aphids were kept in the dark at 20° C. for 24 ± 1 hr.; thus they had been flight mature for about 11 hr. when used in the experiments (Taylor, 1957).

METHODS

General procedure

Of the nine main experiments, nos. 1–5 assessed fat consumption during flight and 6–9 glycogen consumption. Fat and glycogen contents of unflown (control) and flown aphids were estimated chemically. To eliminate variations in fuel content caused by host condition or aphid age, control and flown insects in each experiment were from the same plant and of the same age (± 1 hr.).

Initial fat contents of flown aphids were found by interpolation of graphs relating fat content to live weight in the controls. The differences between these values and

the observed final fat contents represented the amounts used. To assess the amount of glycogen consumed, absolute glycogen contents of flown and unflown insects were compared.

Weighing methods

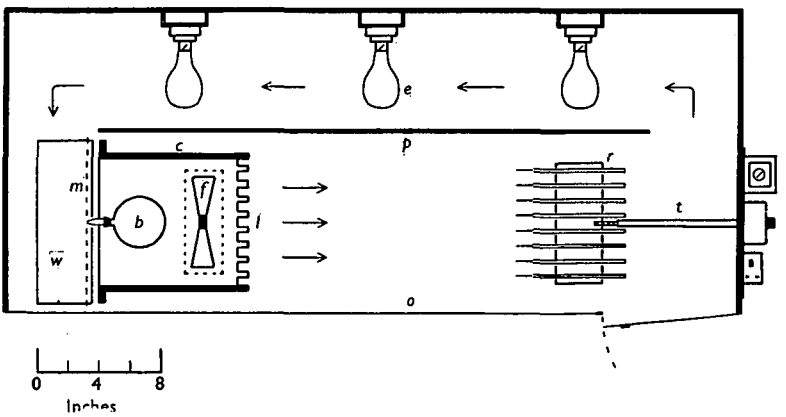
(*a*) *Individual aphids.* Aphids to be flown in Expts. 1–5 were temporarily immobilized by cooling, and the mean of three successive weighings (to 0·01 mg.) taken. Each aphid was then placed in a numbered tube in darkness at 15–17° C. and allowed ½–1 hr. to recover before being tethered for flight. Control aphids were killed with ethyl acetate vapour and immediately weighed and grouped into batches of ascending weight.

(*b*) *Batches of aphids.* Controls in Expts. 6–9 and flown aphids in Expts. 1–9 were weighed in batches immediately after exposure to ethyl acetate vapour.

Flying method

The aphids were tethered for flight by attaching them by the dorsum of the thorax to fine pins with water-colour paint (see Johnson, 1958). All experiments were done in a flight chamber (Text-fig. 1), illuminated from above and heated by nine 60 W. electric lamps. A 6 in. Vent-Axia fan circulated air within the chamber and gave an air current of 1 m.p.h. in the working section. Temperature was controlled at 25–26° C. by a Sunvic thermostat and humidity maintained at 57–82 % R.H. by an evaporator at the fan intake. The pin holders were placed on a three-tiered rack.

Five to twenty aphids were flown simultaneously facing into the air stream; those repeatedly refusing to fly during the first ½ hr. were rejected.



Text-fig. 1. Plan of the flight chamber: *b*, water bottle; *c*, fan casing; *e*, electric lamp; *f*, fan; *l*, louvres; *m*, muslin sheet; *o*, observation panel; *p*, partition; *r*, rack with mounting pins; *t*, thermostat; *w*, water bath.

Analytical methods

(*a*) *Fat determination.* 'Fat', estimated as total ether extractive, includes neutral fats, fatty acids and other ether-soluble material, some of which is valueless as energy reserve.

Batches of aphids were dried at 70° C. to constant weight and then kept at 38° C. for 48 hr. in extraction tubes with 5 ml. petroleum ether (b.p. 40–60° C.) which was

changed approximately every 6 hr. The insects were then dried and re-weighed. The weight of ether-soluble material was thus given by subtraction, not by direct weighing of the extract. Increasing the extraction time to 96 hr. did not increase the amount extracted.

The insects were kept intact by transferring them with a single hair and changing the solvent with a micro-pipette; error from loss of appendage parts was negligible. A minimum of 0·2 mg. fat was aimed at for each determination in unflown insects. Ten aphids were therefore used in each control batch and wherever possible in the flown batches.

(*b*) *Glycogen determination.* Glycogen was estimated by the method of Seifter, Dayton, Novic & Muntwyler (1950) for tissues of low glycogen content, modified as follows: (1) After digestion in 30% KOH the material was filtered through a micro-sintered glass filter (porosity 5–10 μ) and the residue washed in a further quantity of KOH. Filtration was required because of much insoluble material which could not be centrifuged down. (2) The sedimented glycogen was dissolved in 1 ml. distilled water to which 2 ml. of anthrone reagent were added. Thus, a reaction mixture of 3 ml. was tested as against 15 ml. in the original method.

Amounts of chitin similar to that in the insects gave no visible colour when treated and no absorption at 620 mμ, so there was no interference from chitin. As a check on glycogen loss, however, known amounts of glycogen were taken through the same procedure. Three experiments at each of five glycogen levels (0·01–0·05 mg.) gave recoveries of 80·0–94·0%, with a mean of 85·5%. Most of the 14·5% loss probably occurred during filtration and in drainage of the glycogen precipitate; the results of analyses of aphids were corrected for a loss of this magnitude.

The method was sensitive enough (practical range under above conditions 0·002–0·05 mg.) to allow batches of 5 aphids to be used. However, more were usually taken, i.e. 5, 10 or 20 in each control and about 10 in each flown batch.

Histochemical methoas

All insects were decapitated before fixation. To demonstrate glycogen, insects were fixed in Carnoy's, Bouin's or Bouin–Doboscq's fluid, embedded in paraffin and celloidin, and sectioned at 8 μ. Sections were stained in Lugol's solution, Best's carmine, or by the periodic acid-Schiff technique (see Carleton & Drury, 1957). For fats, the insects were fixed in Baker's formaldehyde-calcium, embedded in gelatine and sectioned at 15 μ. Sections were stained in Sudan Black B.

Mass of the flight muscles

It is impracticable to dissect out and weigh the flight muscles of *Aphis fabae*, so the following method was adopted using unflown 24 hr. old alatae from culture. Aphids were serially sectioned and the area occupied by muscle and other tissues in each section of the thorax measured; the volume of muscle as a percentage of the total thoracic volume was then computed. The thoraces of other aphids were isolated and weighed. The weight of the muscles was then calculated using the volume percentage as a weight percentage, assuming that the density of muscle is of the same order as that of other thoracic tissues.

The calculated muscle weights are possibly too high, because the muscle density is

less than that of the rest of the thorax, which includes cuticle. The high density of the cuticle, however, will tend to be counterbalanced by the low density of the fat-body cells. The method is probably at least as accurate as that used by Chadwick & Gilmour (1940) to determine flight-muscle weight in *Drosophila*—by subtracting the dry weight of the thoracic skeleton, after treatment with KOH, from the weight of the freshly killed thorax.

RESULTS

Flight performances

Different aphids behaved differently during tethered flight. Some stopped flying for no apparent reason, only to re-start within a few seconds; others stopped occasionally and required re-stimulation; others flew continuously for periods of 5–8 hr. Those aphids that stopped and failed to re-start spontaneously were induced to fly again by stimulating their tarsi, or, by passing them rapidly forward through the air.

Aphids were flown for set times of up to 6 hr. in Expts. 1–9, and to apparent exhaustion in Expts. 10 and 11. In the latter experiments, flight usually became intermittent as the aphids became fatigued, but there was generally no sharp end-point to flight (cf. Johnson, 1955). An aphid was considered exhausted therefore when it ceased to fly, and: (1) it could not be induced to fly again, even after a rest of *c.* 1 min. (39 % of the insects flown in Expts. 10 and 11); or (2) stimulation produced only a mere vibration of the wings (5 %); or (3) after further stimulation, three successive flights each lasted less than 1 min. (56 %).

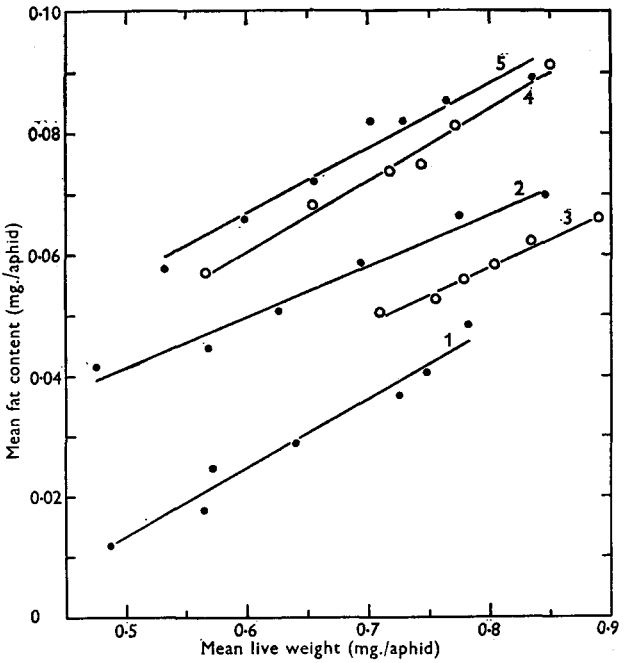
Fuel content before flight

(*a*) *Fat content.* The 24 hr. old unflown aphids reared in the laboratory on broad bean contained least fat (Expt. 1), the mean proportion of fat to live weight being 4·4 % (15·6 % of the dry weight). Aphids from a natural infestation of a field bean (Expt. 5) contained most, with 11·1 % of the live weight (31·4 % of the dry weight). Within each experiment the mean weight of fat per aphid was highly correlated with live weight; separate regressions for the two variables were fitted for each series of controls (Text-fig. 2). On the average, positive differences of 0·1 mg. live weight were associated with positive differences of 0·008–0·012 mg. fat/aphid. The positions of the regression lines demonstrate the different amounts of fat in the insects from the various hosts, differences that probably reflect the nutritional value of the sap imbibed by the aphids during development.

(*b*) *Glycogen content.* The aphids analysed for glycogen were from culture on broad beans (Expts. 6–9). The glycogen constituted between 0·5 and 1·0 % of the live weight of the insects (1·7–3·4 % of the dry weight). Glycogen content and aphid live weight were not significantly correlated ($r = 0{\cdot}066$; $P > 0{\cdot}10$) (Text-fig. 3).

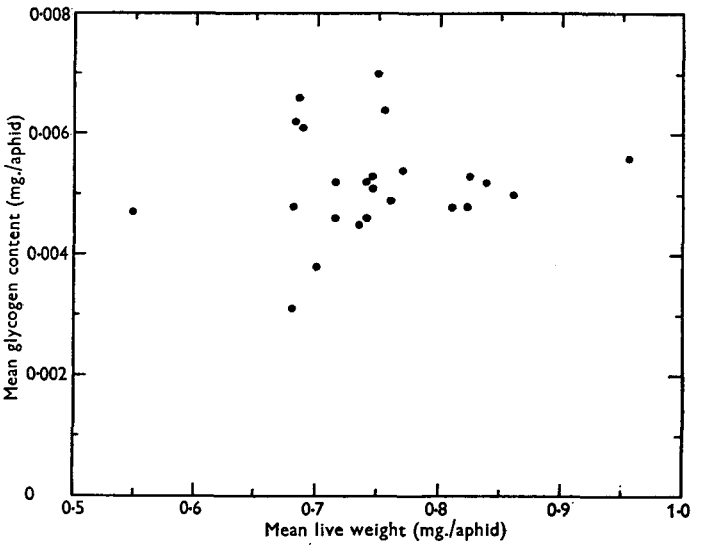
Fuel utilization during flight

(*a*) *Fat utilization.* Table 1 gives the mean fat contents of batches of aphids before and after flight: pre-flight fat contents were estimated by interpolation of the appropriate regression in Text-fig. 2 (mean wt. of fat/aphid); pre- and post-flight live weights are given elsewhere (Table 2, Cockbain, 1961). Differences between initial and final fat represents the amounts used during flight.



Text-fig. 2. Relationship between mean fat content and live weight in unflown 24 hr. old alatae from different host plants:

Expt. no.	Host plant	Location	Corr. coeff. (r)	Reg. coeff. (b)
1	Broad bean	Culture	+0·968	+0·115
2	Spindle	Field	+0·986	+0·084
3	Dock	Field	+0·989	+0·092
4	Broad bean	Field	+0·985	+0·118
5	Field bean	Field	+0·972	+0·106



Text-fig. 3. Relationship between mean glycogen content and live weight in unflown 24 hr. old alatae.

Table 1. *Fat contents of batches of aphids before and after flight (Expts. 1–5)*

Flight duration (hr.)	Aphid condition	Expt. 1 No. of aphids	Expt. 1 Weight of fat (mg.)	Expt. 2 No. of aphids	Expt. 2 Weight of fat (mg.)	Expt. 3 No. of aphids	Expt. 3 Weight of fat (mg.)	Expt. 4 No. of aphids	Expt. 4 Weight of fat (mg.)	Expt. 5 No. of aphids	Expt. 5 Weight of fat (mg.)
1	Before flight	10	0·24	9	0·50	8	0·50	7	0·54	11	0·81
	After flight	10	0·23	9	0·52	8	0·475	7	0·51	11	0·825
2	Before flight	8	0·15	8	0·43	9	0·52	8	0·64	8	0·61
	After flight	8	0·12	8	0·365	9	0·45	8	0·535	8	0·555
4	Before flight	10	0·30	8	0·44	9	0·54	8	0·66	8	0·60
	After flight	10	0·17	8	0·345	9	0·38	8	0·505	8	0·47
6	Before flight	8	0·30	7	0·47	9	0·53	7	0·57	7	0·55
	After flight	8	0·125	7	0·295	9	0·23	7	0·365	7	0·405

Table 2. *Analysis of variance of mean decreases in fat content during flights of different duration (Expts. 1–5)*

Flight duration (hr.)	1	2	4	6
Mean decrease (mg. fat/aphid) (±0·00103) ...	0·0010	0·0080	0·0156	0·0260

Source of variance	D.F.	Mean square
Between hosts (expts.)	4	0·0000353**
Between flight durations	3	0·0005738***
Residual	12	0·0000053

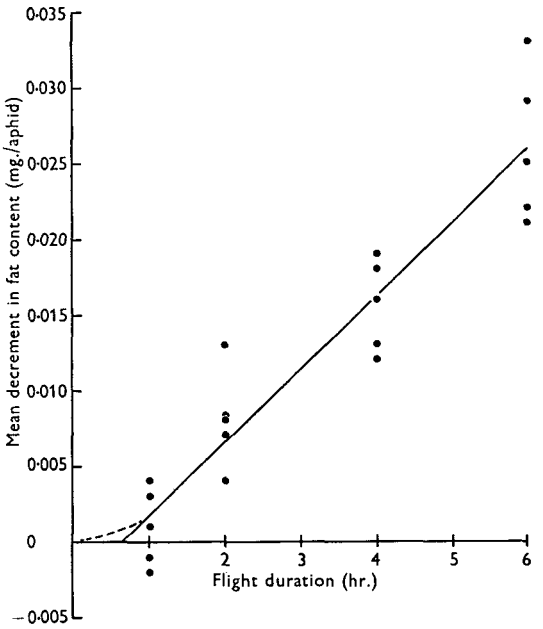
** $P = 0{\cdot}01$–$0{\cdot}001$. *** $P < 0{\cdot}001$.

Decreases in fat, recorded in all but two of the batches, represented 27–58 % of the initial amount in aphids flown for 6 hr. The analysis of variance (Table 2) shows that no significant decrease had occurred after a flight of 1 hr., but the mean decreases after 2, 4 and 6 hr. were all significant. The significant difference between the amounts of fat used in the different experiments, shown by the analysis, was possibly caused by differences in insect weight and 'flight intensity'.

Text-fig. 4 shows mean estimated decrements in fat content during flight. After the first hour, the average rate of fat consumption was 0·005 mg./aphid/hr. The absence of a significant decrease during the first hour, and the fact that the regression line cuts the abscissa before the origin, suggests that a different fuel was used during the early period of flight.

(*b*) *Glycogen utilization.* Text-fig. 5 (graph A) shows the mean glycogen contents of aphids after flights of $\frac{1}{4}$–5 hr. The curve fitted to the mean values shows a high rate of loss during early flight, with a mean decrement of 0·0028 mg./aphid during the first hour and 0·0006 mg. during the second. The decrease during flight was highly significant, a significant decrease being recorded even after $\frac{1}{2}$ hr. (Table 3).

(*c*) *Effect of excretion.* The frequency of excretion of a batch of aphids during flight follows a course similar to the rate of glycogen decrease, i.e. it is highest early in flight. In view of possible interference from gut carbohydrates in the glycogen estimations (anthrone procedure), the possibility of errors from excretion were tested. The number of honeydew droplets excreted during flight and the post-flight glycogen contents of the flown aphids were compared with those of aphids starved (without flight) for equivalent periods at the same temperature (Table 4 and Text-fig.



Text-fig. 4. Decrease in fat content during flight at 25–26° C. $b = +0{\cdot}0048$.

Table 3. *Analysis of mean decreases in glycogen content during flights of different duration* (*Expts.* 6–9)

Flight duration (hr.)	0	¼	½	1	2	3	5
Mean glycogen content (mg./aphid)	0·0052	0·0041	0·0036	0·0024	0·0018	0·0010	0·0009
S.E.	±0·00017	—	—	±0·00041	—	—	—
Mean decrease (mg. glycogen/aphid)*	—	0·0011	0·0016	0·0028	0·0034	0·0042	0·0042
S.E.	—	—	—	±0·00044	—	—	—

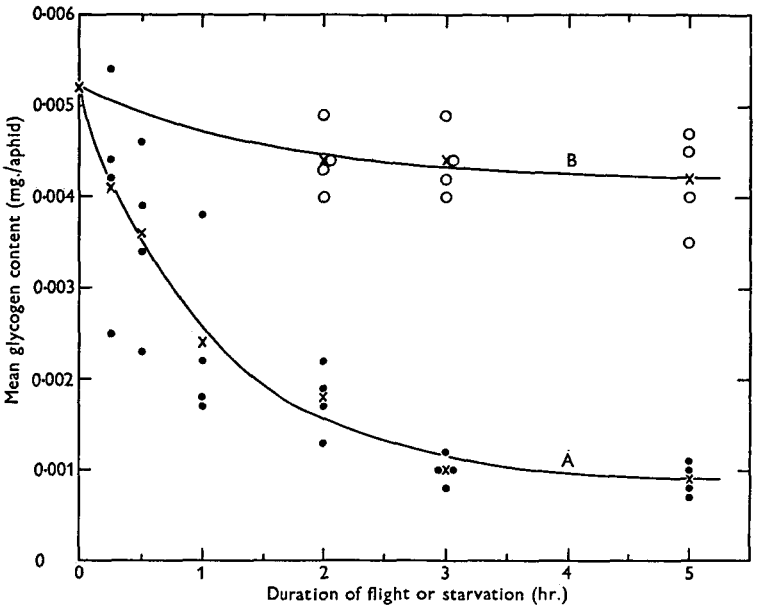
* Decrease significant ($P = 0{\cdot}02$) when greater than 0·0013, i.e. 0·00044 × 3.

graph B). Excretory droplets were collected on pH indicator paper. Droplet sizes were similar in both flight and starvation experiments.

Starved aphids excreted more than flying aphids but their glycogen contents decreased only slightly. The mean decrease during 5 hr. (0·001 mg./aphid) was similar to that which occurred during a ¼ hr. flight and was probably brought about by muscular activity, e.g. walking, during the experiment. Thus the diminution in glycogen during flight represented the amounts utilized.

Rates of metabolism during flight

Approximate metabolic rates were calculated from the mean amounts of fat used in Expts. 1–5 (Text-fig. 4) and glycogen in Expts. 6–9 (Text-fig. 5) using the conversion figures given by Carpenter (1939); the two batches in which there was no decrease in fat have been omitted from the calculations. The rates are given in Table 5 with the calculated contributions of fat and glycogen to the total amount of energy transformed.



Text-fig. 5. Glycogen contents after different durations of (A) flight and (B) starvation at 25–26° C. ×, mean values.

Table 4. *Excretory activity of alatae during flight and starvation at* 25–26° C.

Period of flight or starvation (hr.)	Flight		Starvation	
	No. of aphids	Droplets/aphid	No. of aphids	Droplets/aphid
2	38	0·79	30	1·43
3	36	0·86	30	1·57
5	37	0·97	20	1·75

Table 5. *Rates of metabolism during tethered flight and contributions of fat and glycogen to the total amount of energy transformed*

Flight duration (hr.)	Metabolic rate		Energy derivation	
	Energy expenditure (cal./g./hr.)	Oxygen consumption (ml./g./hr.)	From glycogen (%)	From fat (%)
1	52·2	10·8	30	70
2	65·9	13·8	16	84
4	59·5	12·5	10	90
6	57·7	12·2	7	93

The mean rate was 59 cal./g. live wt./hr. or 12 ml. O_2/g./hr. At this rate, the maximum amount of glycogen in the aphids from culture would maintain flight for 42 min., i.e. 0·001 mg. glycogen if used exclusively would maintain a 6 min. flight; the mean flight duration of *Drosophila melanogaster* on 0·001 mg. glucose is 6·3 min. (Wigglesworth, 1949). Thus over 8 times the maximum amount of glycogen so far recorded would be required for a 6 hr. flight if glycogen alone was available. Possibly the aphids from natural infestations contained, and used, greater amounts of glycogen than those from culture, and the metabolic rates and contributions of glycogen were greater tha

those calculated. The available evidence, however, suggests that, at least after the first hour, fat provides most of the energy for flight, i.e. *c.* 90% of the energy for a tethered flight of 6 hr.; the corresponding value for the locust *Schistocerca gregaria* during a flight of 5 hr. is 80–85% (Weis-Fogh, 1952).

Most of the metabolism during flight is in the flight muscles. The thorax constituted between 30·5 and 38·3% of the live body weight of the 24 hr. old insects (mean = 35·1%, $n = 9$) and the flight muscles occupied between 34·5–39·2% of the total volume of the thorax (mean = 37·0%, $n = 5$). Assuming that the density of the muscle tissue was similar to that of the remaining thoracic tissues, the flight muscles constituted *c.* 13% of the live body weight (cf. 18% in *Drosophila repleta*, Chadwick & Gilmour, 1940, and 13% in *D. melanogaster*, Hocking, 1953).

From this value, the approximate rates of metabolism of the muscles during flight in Expts. 1–5 were calculated; these varied between 400 and 500 cal./g. muscle/hr. Assuming that the proportion of water in the muscles was the same as that in the body as a whole (mean = 68%), the mean rate of fuel consumption was 17% of the dry muscle weight/hr.

Flight capacity in relation to initial fat content

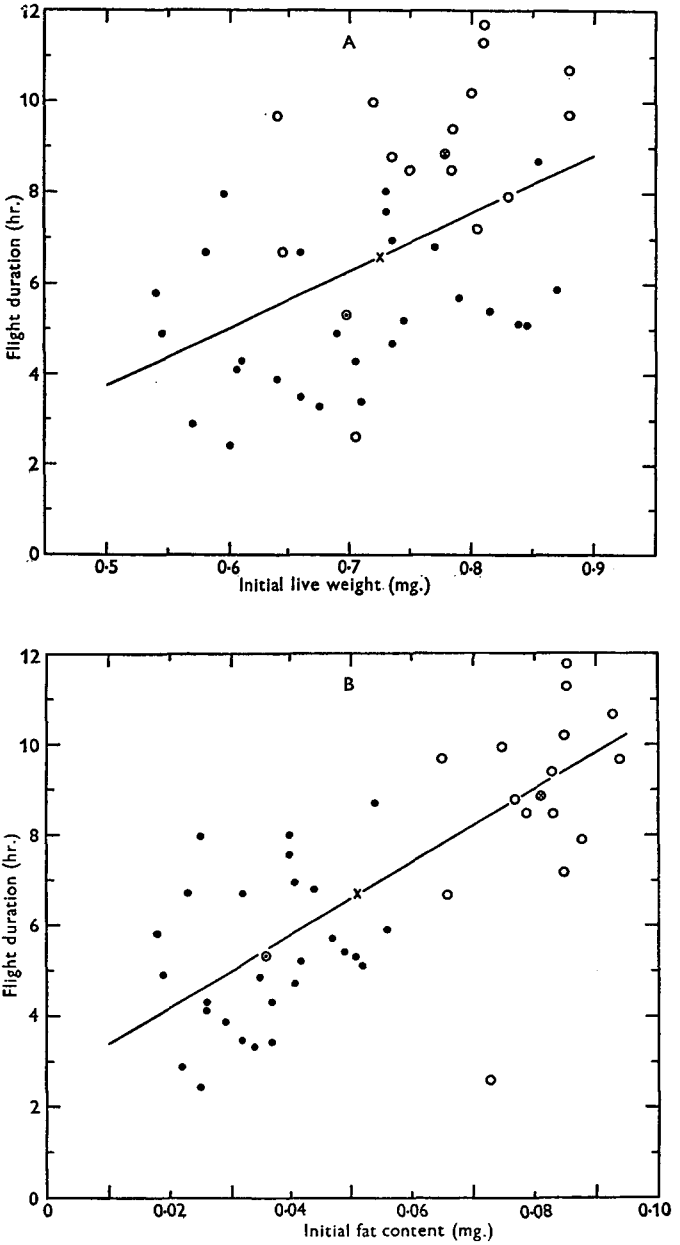
Two experiments (10 and 11) were made on flight capacity using aphids from the same sources as those used in Expts. 1 and 4 respectively, i.e. broad beans in culture and in the field. Aphids were weighed individually before flight and their initial fat contents estimated by interpolation of the appropriate control graph in Text-fig. 2; the values are only approximate for the regressions in Text-fig. 2 are based on mean, not individual, weights.

The mean flight duration was 5·3 hr. in Expt. 10 (culture aphids) and 8·9 hr. in Expt. 11 (field aphids). Text-fig. 6A, B show the durations plotted against initial live weight and fat content respectively. A slight correlation was found between duration and fat content in Expt. 10 ($r = +0{\cdot}324$; $P = 0{\cdot}10$), but none between duration and fat content in Expt. 11, nor between duration and initial live weight ($P > 0{\cdot}10$). The combined data, however, give highly significant correlations between duration and live weight ($r = +0{\cdot}490$; $P = 0{\cdot}001$) and between duration and fat content ($r = +0{\cdot}784$; $P = 0{\cdot}001$). When flight duration is considered in relation to initial live weight, the results from Expt. 10 (aphids with low fat contents) lie mainly below the common regression line, and those from Expt. 11 (aphids with high fat contents) above. When duration is plotted against initial fat content, however, a regression continuous from group to group is obtained.

The available evidence therefore suggests that flight capacity is limited by initial fat content; had other processes, e.g. neural fatigue, accumulation of waste metabolites, water loss (see Cockbain, 1961), etc., constituted limiting factors to flight, high correlation between flight duration and initial fat content in aphids from two sources would have been unlikely.

It may be calculated from the data in Table 3 that after a flight of 5·3 hr. aphids from culture would have used a mean of 51% of their initial fat, and after a flight of 8·9 hr. those from broad bean in the field would have used 56%. Thus in both experiments the mean proportion of initial fat used during flight was about the same, but in neither was exhaustion associated with entire depletion of fat. Some of the remaining 'fat' (ether extract) will not be fuel reserve, however, and the rest, in parts of the body

remote from the flight muscles (see next section), may not have been mobilized quickly enough to support wing movement (cf. mobilization of glycogen in *Drosophila*, Wigglesworth, 1949).



Text-fig. 6. Flight capacity at 25–26° C. in relation to (A) initial body weight ($b = +12{\cdot}67$) and (B) initial fat content ($b = +75{\cdot}13$). ●, Culture aphids; ⊙, mean for culture aphids; ○, field aphids; ⊗, mean for field aphids; ×, common mean.

Distribution of the flight reserves

General anatomy of an alate aphid is shown in Pl. 1 A, and histology of the thoracic fat body in Pl. 1 B.

(*a*) *Distribution of fat.* Most of the fat lies in the fat-body cells of the thorax and abdomen. In the thorax the fat body surrounds all the organs; in the abdomen it is mainly restricted by the presence of embryos to the lateral and posterior regions. Typically the fat occurs as globules within the cytoplasmic network of the cells, but in unflown insects the staining of fat in many cells is so intense that the globular nature is masked. Most cells in the thorax are free from fat after prolonged flight to exhaustion (cf. Pls. 1 C, D, 2 E, F) but small deposits persist in the abdomen.

Fat also occurs between the fibrils of the indirect flight muscles, but no discrete droplets were detected. The muscle fat becomes less during flight, but the muscles stain for fat even when the insect has flown to apparent exhaustion (cf. Pl. 2 E, F).

(*b*) *Distribution of glycogen.* Glycogen was sparsely distributed and was principally in the fat body. Glycogen occurs in fixed material as small flakes, mainly around the periphery of the fat-body cells. It also occurs along the surface of the muscle fibres but none was detected within or between the fibrils. Glycogen was barely detectable in thorax or abdomen of insects that had flown for long periods (cf. Pl. 2 G, H).

SUMMARY

1. Fat contents (ether extracts) of unflown 24 hr. old alatae of *Aphis fabae* Scop., from different host plants, range from 3–12 % of the live weight and 9–33 % of the dry weight. Glycogen contents of alatae reared in culture range from 0·5–1 % of the live weight and 1·7–3·4 % of the dry weight.

2. Both fat and glycogen are consumed during tethered flight. Glycogen is used during early flight and fat is the principal fuel after the first hour, when it is consumed at a mean rate of 0·005 mg./aphid/hr. and provides about 90 % of the energy for a 6 hr. flight. The amounts of glycogen in laboratory-reared aphids alone could not maintain flight for more than $\frac{3}{4}$ hr.

3. Metabolic rates during tethered flight range from 52 to 66 cal./g. live wt./hr. or 11–14 ml. O_2/g./hr. The flight muscles constitute about 13 % of the live body weight of 24 hr. old aphids, and, attributing most of the metabolism during flight to these muscles, their metabolic rates range from 400 to 500 cal./g./hr.

4. Flight capacity of 24 hr. old aphids at 25–26° C. is directly related to initial fat content and varies between 3 and 8 hr. in aphids from culture (mean of 4 % fat by live weight) and between 7 and 12 hr. in aphids from the field (10 % fat). Flight fatigue occurs before all the fat reserves are used; possibly fat stored in parts of the body remote from the flight muscles cannot be mobilized rapidly enough to support continuous flight.

5. Fat and glycogen reserves occur mainly in the fat-body cells of the thorax and abdomen; fat also occurs between the fibrils of the indirect flight muscles and glycogen along the surface of the fibres. Flight-exhausted insects have little or no fat in the thorax, but small deposits remain in the abdomen; little glycogen can be detected in culture aphids flown to exhaustion.

It is a pleasure to express my thanks to Dr C. G. Johnson for his supervision and helpful criticism of this work, and to Mr L. R. Taylor and Dr K. Mellanby for reading the manuscript. My thanks are due also to Mr C. I. Carter for technical assistance; to Mr J. H. A. Dunwoody for the statistical analyses in Tables 2 and 3; and to Mr F. D. Cowland for taking the photomicrographs.

REFERENCES

CARLETON, H. M. & DRURY, R. A. B. (1957). *Histological Techniques*, 3rd ed. London: Oxford University Press.

CARPENTER, T. M. (1939). Tables, factors, and formulas for computing respiratory exchange and biological transformations of energy, 3rd ed. *Publ. Carneg. Instn*, no. 303 B.

CHADWICK, L. E. & GILMOUR, D. (1940). Respiration during flight in *Drosophila repleta* Wollaston: the oxygen consumption considered in relation to the wing-rate. *Physiol. Zoöl.* **13**, 398–410.

COCKBAIN, A. J. (1961). Water relationships of *Aphis fabae* Scop. during tethered flight. *J. Exp. Biol.* **38**, 175–80.

HOCKING, B. (1953). The intrinsic range and speed of flight of insects. *Trans. R. Ent. Soc. Lond.* **104**, 223–345.

JOHNSON, B. (1955). Studies in flight, host selection and reproduction in aphids. Thesis (Ph.D.), London University.

JOHNSON, B. (1958). Factors affecting the locomotor and settling responses of alate aphids. *Anim. Behav.* **6**, 9–26.

SEIFTER, S., DAYTON, S., NOVIC, B. & MUNTWYLER, F. (1950). The estimation of glycogen with the anthrone reagent. *Arch. Biochem.* **25**, 191–200.

TAYLOR, L. R. (1957). Temperature relations of teneral development and behaviour in *Aphis fabae* Scop. *J. Exp. Biol.* **34**, 189–208.

WEIS-FOGH, T. (1952). Fat combustion and metabolic rate of flying locusts (*Schistocerca gregaria* Forskål). *Phil. Trans.* B, **237**, 1–36.

WIGGLESWORTH, V. B. (1949). The utilization of reserve substances in *Drosophila* during flight. *J. Exp. Biol.* **26**, 150–63.

EXPLANATION OF PLATES

PLATE 1

A. Sagittal section of one-day-old alate *Aphis fabae*. *c*, cauda; *dlm*, dorsal longitudinal flight muscle; *dvm*, dorso-ventral muscle; *e*, embryos; *f*, fat body; *m*, mid-gut; *t*, thoracic ganglion. PAS (after saliva treatment) and Mayer's haemalum, × 75.

B. Section of thoracic fat body of 6-day-old aphid showing cytoplasmic network, nuclei and fat vacuoles within the cells. Heidenhain's iron-haematoxylin, × 470.

C. One-day-old unflown aphid. Section of thorax beneath meso-scutellum stained for fat. Streaming appearance of the fat is a sectioning artifact. Sudan Black B and carmalum, × 470.

D. As for C, but flown for 5½ hr.

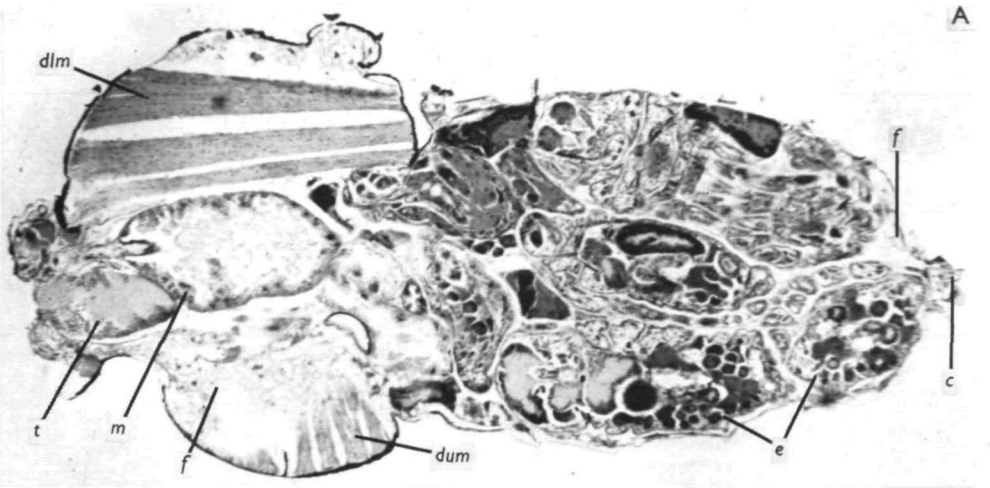
PLATE 2

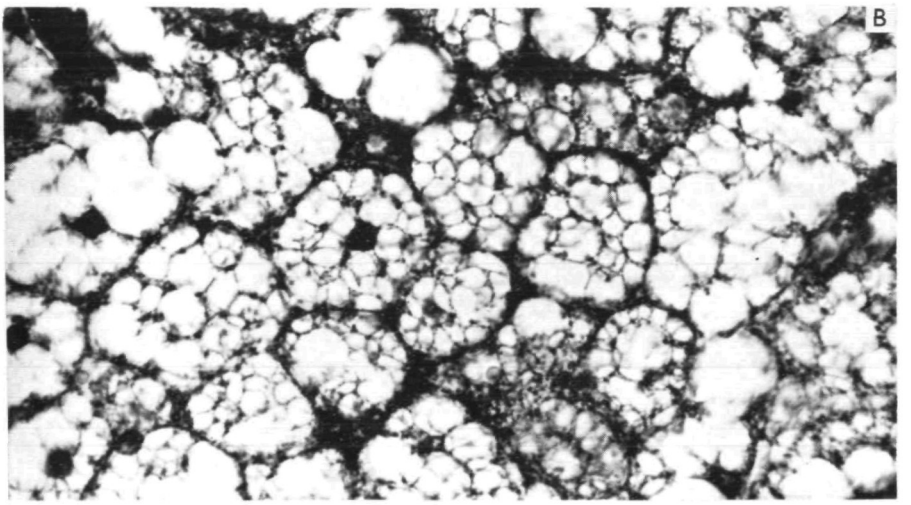
E. One-day-old unflown aphid. Section of fat body and dorso-ventral muscle stained for fat. Sudan Black B and carmalum, × 170.

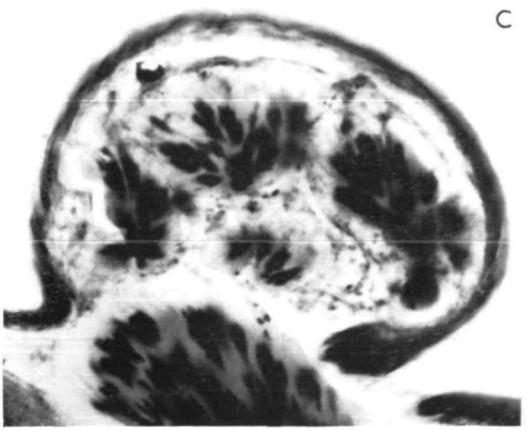
F. As for E, but flown for 8 hr.

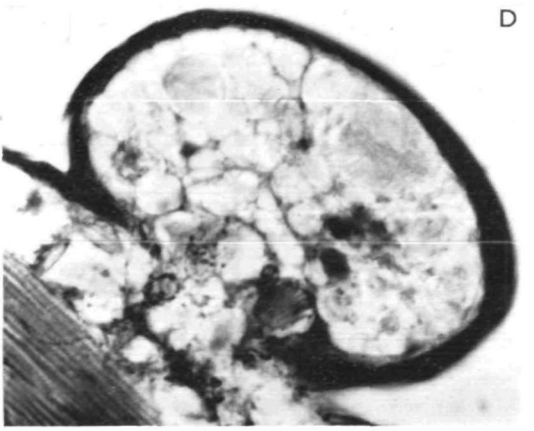
G. One-day-old unflown aphid. Section of cauda stained for glycogen (*g*). PAS, × 470.

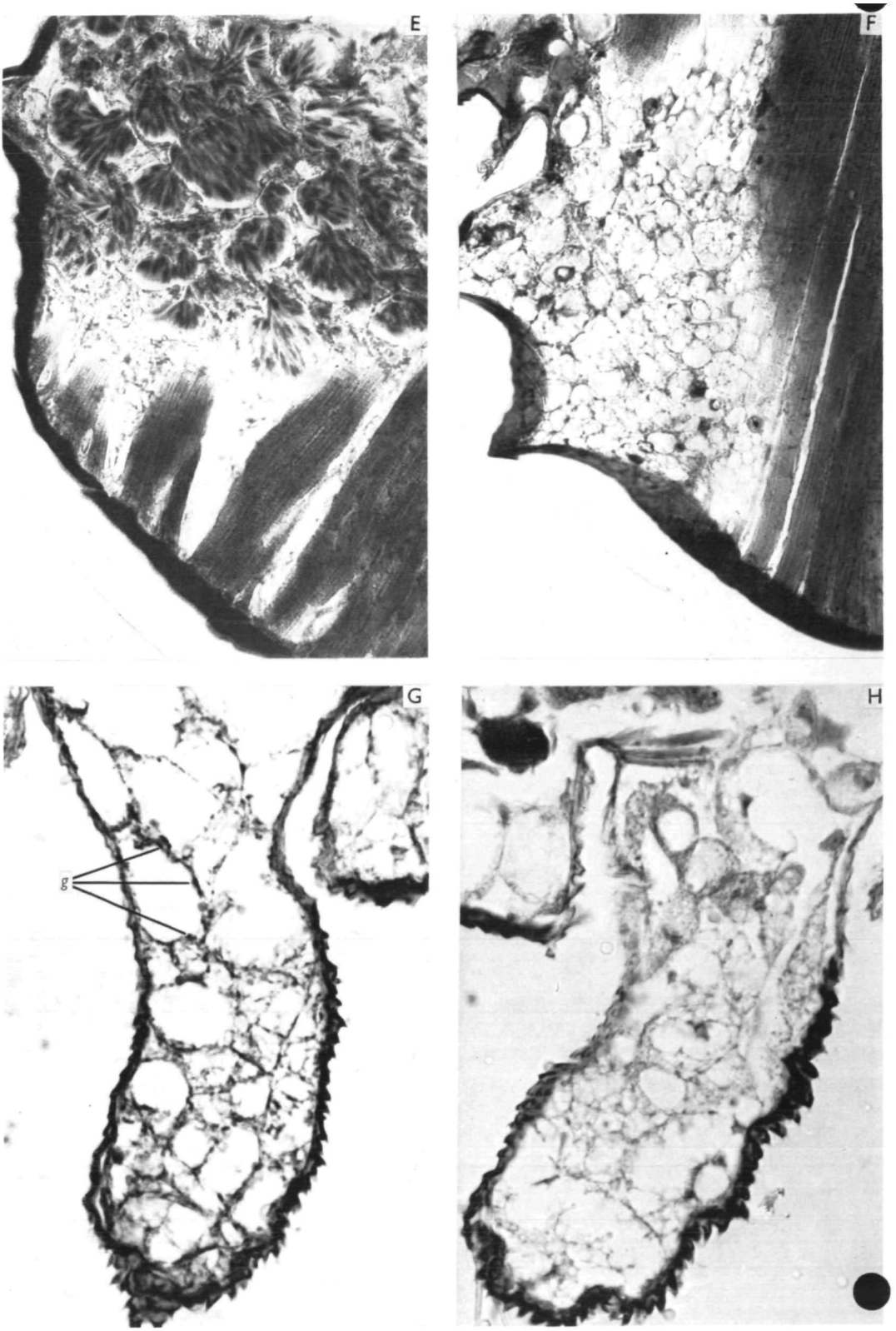
H. As for G, but flown for 12 hr.


A
dlm
f
t
m
f
dum
e
c


B


C


D



A. J. COCKBAIN