THE UTILIZATION OF RESERVE SUBSTANCES IN *DROSOPHILA* DURING FLIGHT

By V. B. WIGGLESWORTH
Agricultural Research Council, Unit of Insect Physiology, Department of Zoology, University of Cambridge

(*Received 22 December* 1948)

(With Four Text-figures)

The respiratory quotient in *Drosophila* during flight is about 1·0 (Chadwick, 1947), and the duration of flight to exhaustion varies with the age of the fly in the same manner as does the total glycogen—increasing steeply during the first 5 days of adult life, remaining at a maximum for about 10 days and then falling gradually until the fly is 5 or 6 weeks old (Williams, Barness & Sawyer, 1943). It appears certain that glycogen is the main or sole reserve substance consumed during flight.

It is the object of the present work to provide a histological picture of the distribution of the reserve substances and their mobilization during sustained flight. In the course of the work it has been possible to compare directly the efficiency of different substances as sources of energy for flight. *D. melanogaster* has been used throughout. The insects have been reared and maintained on standard maize-meal medium at 25° C.

DISTRIBUTION OF GLYCOGEN IN THE MATURE FLY

For the demonstration of glycogen the flies have been fixed in Carnoy's solution, transferred to absolute alcohol and split into two halves by a median longitudinal cut. The half insects have been stained in a saturated solution of light green in 90% alcohol and the glycogen revealed (i) by the iodine method as previously described (Wigglesworth, 1942), (ii) with Best's carmine. The two methods give identical results. Sections of Carnoy-fixed material have been cut in paraffin and celloidin and stained with Best's carmine.

Fig. 1 shows the general distribution of glycogen in the mature fly 1 week after emergence, and the details of its deposition in the cells.

(i) *Fat body*. The bulk of the glycogen is in the fat body of the abdomen. The fat-body cells have a few fat droplets around the nuclei, and occasional vacuoles with clear watery contents, but for the most part they are filled with huge peripheral deposits of glycogen (Fig. 1 J). Cells stained deeply with haematoxylin show the appearance seen in Fig. 1 H, with a cytoplasmic network ramifying through the glycogen deposits. Similar fat-body cells more or less laden with glycogen occur in the head, at the base of the legs, along the sides of the thorax, and in the scutellum.

(ii) *Halteres*. The next most conspicuous deposit of glycogen is in the large clear

cells which fill the knob of the haltere (Fig. 1 D). These are almost certainly modified fat-body cells. They show ramifying strands of cytoplasm between the dense glycogen deposits like the fat-body cells but they contain almost no fat (Fig. 1 E).

(iii) *Flight muscles.* The third important location for glycogen is in the indirect flight muscles. This glycogen varies in amount but is usually less, or at least less

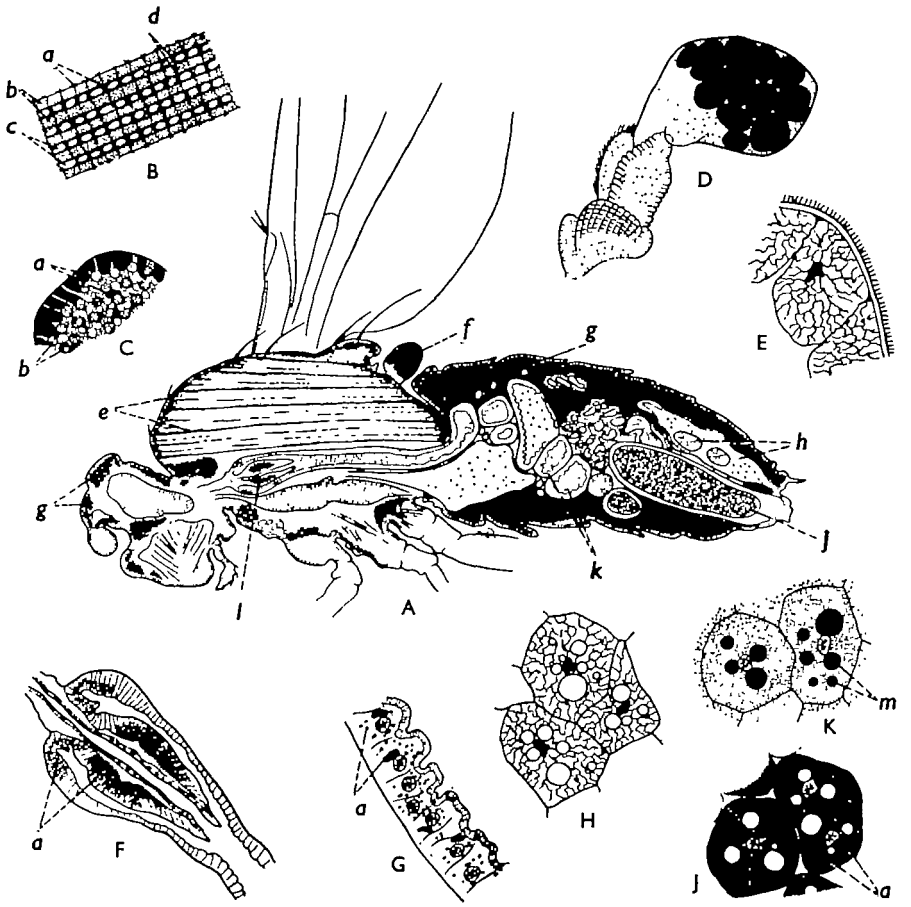

Fig. 1. Distribution of glycogen in the mature fly. A, sagittal section of female *Drosophila*, glycogen shown in black. B, longitudinal section of indirect flight muscle. C, transverse section through margin of fibril bundle in flight muscle. D, haltere. E, section through wall of haltere knob showing detail of the glycogen-containing cells. F, longitudinal section of proventriculus. G, section through wall of mid-gut. H, fat-body cells showing cytoplasmic network and fat vacuoles. J, fat-body cells showing glycogen. K, fat-body cells showing fat. *a*, glycogen; *b*, fibrils; *c*, sarcosomes; *d*, sarcoplasm; *e*, glycogen between muscle bundles and insertions; *f*, glycogen in haltere; *g*, fat body; *h*, rectum and rectal glands; *j*, oocyte with glycogen; *k*, mid-gut; *l*, proventriculus with glycogen; *m*, fat.

conspicuous, than the massive deposits in the halteres. It occurs along the surface of the muscle masses, and around their points of insertion. Each muscle mass is made up of some ten to thirty lesser bundles each containing fifty to one hundred fibrils. Lesser deposits of glycogen occur between these smaller bundles. Finally, there are deposits of glycogen between the individual fibrils.

The structure and arrangement of these fibrils will form the subject of a separate paper; but it may be stated here that an oval sarcosome lies opposite each half of each sarcomere. Depending upon the method of staining, either the sarcosome or the intervening plasma may be caused to show up. In sections stained with Best's carmine and chlorazol black the sarcosomes are unstained, appearing as colourless vacuoles, and the glycogen is seen in the form of irregular deposits in the transverse meshwork of sarcoplasm which binds the fibrils together (Fig. 1 B, C). By the methods employed, no glycogen can be detected within the fibrils themselves, nor in the other muscles.

(iv) *Proventriculus.* Fine granules of glycogen can be seen in the cardiac cells of the mid-gut which secrete the chitinous peritrophic membrane (Wigglesworth, 1930; Siang-Hsu, 1947), but there is a far more conspicuous mass which fills the vacuolated cells of the oesophageal invagination—the plug against which the peritrophic membrane is moulded (Fig. 1 F).

(v) *Mid-gut cells.* Lastly there are comparatively small amounts of glycogen in the epithelial cells of the mid-gut (Fig. 1 G). None can be detected by this method in the rectal papillae, Malpighian tubes or oenocytes. There are traces only in the cells of the central nervous system. There are, of course, large amounts in the yolk of the developing eggs.

DISTRIBUTION OF FAT IN THE MATURE FLY

For the demonstration of fat the flies have been fixed in Bouin's aqueous mixture, cut in half as before, washed in 50% alcohol saturated with lithium carbonate and stained with B.Z.L. blue (Ciba). Sections have been prepared from flies fixed in Altmann's fixative and stained with Delafield's haematoxylin or with methylene blue.

Reserves of fat are far less conspicuous than those of glycogen. In the fat body there are clusters of comparatively small droplets around the nuclei (Fig. 1 K); there are small amounts in the epithelial cells of the mid-gut, and occasional very minute droplets occur in the cells of the haltere knobs. There is some diffuse staining of the flight muscles, notably in the sarcosomes, as well as in the other tissues, but no discrete droplets of stored fat.

CHANGES IN THE RESERVES OF GLYCOGEN, FAT AND PROTEIN IN THE YOUNG AND IN THE OLD FLY

In the newly emerged fly the 'larval fat body' is still evident and fills a great part of the abdomen. It is made up of spherical cells or groups of cells which are readily detached from one another (Fig. 2 A'). These cells contain large droplets of fat and refractile spheres of reserve protein.* Glycogen is scanty and takes the form of small peripheral deposits with granules scattered along the radiating strands of cytoplasm. The 'imaginal fat body' is made up of closely packed cells with small drops of fat and glycogen (Fig. 2 A). There is no demonstrable glycogen in the thoracic muscles or in the proventriculus, and there are traces only in the halteres (Fig. 2 D).

* The protein varies greatly in amount; the spheres are most numerous in the larger flies, irrespective of the sex.

During the next 48 hr., the larval fat-body cells diminish in size (Fig. 2B′); their protein droplets disappear, their fat is used up, and by 4 days after emergence no trace of these cells can be found. Meanwhile the adult fat body enlarges rapidly, particularly by the deposition of great quantities of glycogen (Fig. 2B). It reaches the state described in the mature fly in about 4 days (Fig. 2C). Likewise the glycogen in the halteres is gradually built up, reaching its peak at the same time (Fig. 2E, F).

In the fly at 4 or 5 weeks after emergence there is a general reduction in the amount of visible glycogen, but this amount still appears much greater than in the newly emerged fly.

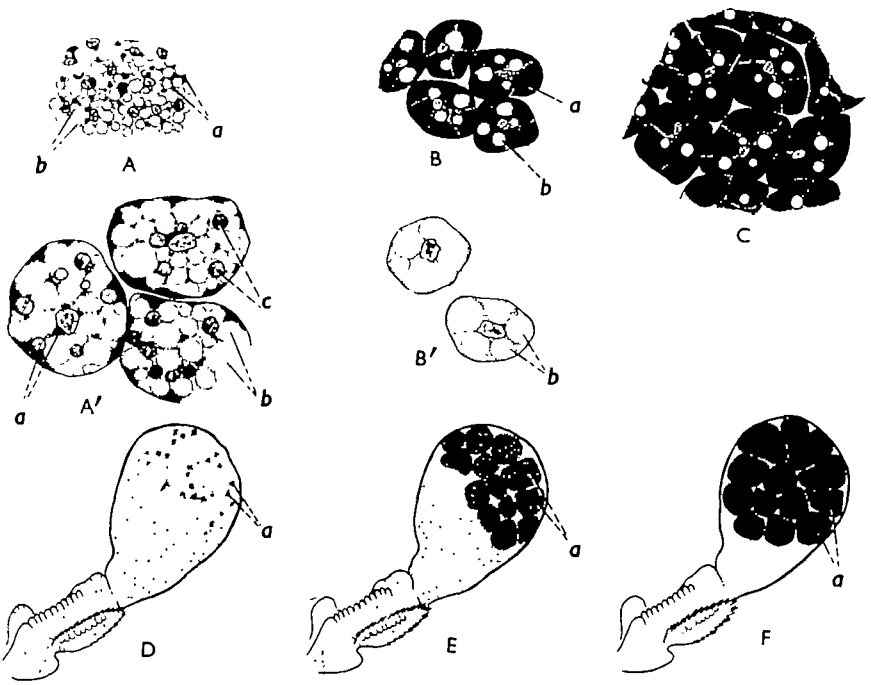

Fig. 2. Accumulation of glycogen in fat body and halteres in the young fly. A, imaginal fat body of newly emerged fly. A′, larval fat body of the same. B, imaginal fat body at 48 hr. B′, larval fat body of the same. C, imaginal fat body at 4 days. D, haltere of newly emerged fly. E, haltere at 48 hr. F, haltere at 4 days, showing progressive increase in glycogen. *a*, glycogen; *b*, fat; *c*, protein spheres.

UTILIZATION OF RESERVES DURING STARVATION

Mature flies, 1 week after emergence, have been given water alone and kept at 25° C. They survive for 2–3 days (cf. Hassett, 1948). By the end of 24 hr., glycogen is becoming markedly reduced in the fat body; reduction in the halteres is not so great, hence these deposits show up more conspicuously. Fat is reduced from the outset, concurrently with the glycogen.

In the later stages of starvation (2–3 days) glycogen disappears completely from the fat body (Fig. 3B), which is greatly reduced in size, and from the halteres and proventriculus. On the other hand, glycogen often persists in the thoracic muscles,

which may contain plentiful deposits even at the time of death. In the moribund fly there are usually a few small scattered droplets of fat still remaining in the fat body (Fig. 3B′). But it is clear that fat and glycogen are both consumed during starvation, at relative rates more or less proportional to the amounts present.

UTILIZATION OF RESERVES DURING FLIGHT

Flies of both sexes have been suspended by securing the dorsum of the thorax with wax to the head of a small entomological pin, using the technique of Hollick (1940). The pin is inserted into the cork of a 2 × 1 in. vial. The suspended insects will fly continuously for long periods. When they stop they are started again by smartly

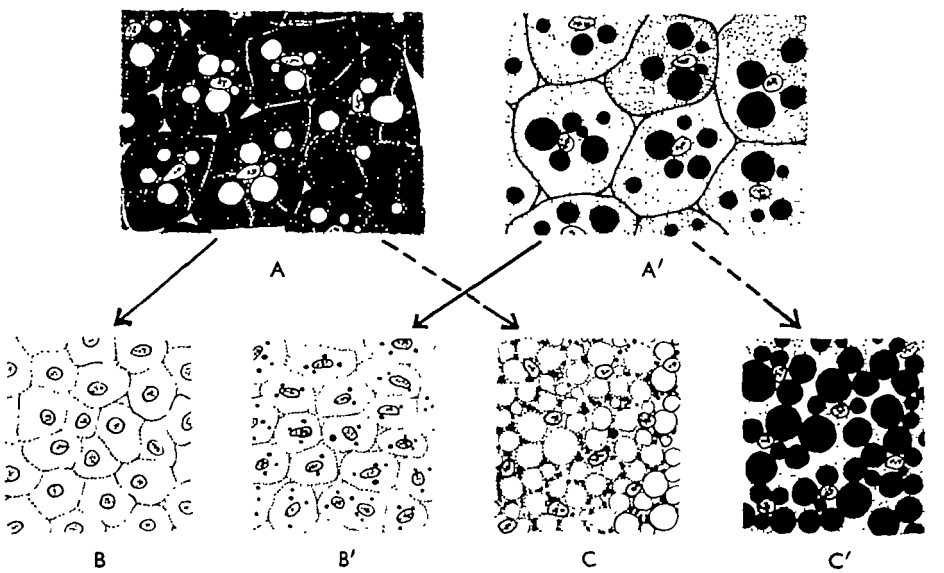

Fig. 3. A, fat-body cells of fly one week old showing glycogen. A′, the same showing fat droplets. B, the same after starvation until moribund; no glycogen remains. B′, the same showing minute fat droplets in the starved fly. C, fat-body cells in fly which had flown to exhaustion: granules of glycogen between the fat vacuoles. C′, the same showing fat droplets.

withdrawing the cork from the tube. Flight was maintained at a temperature of 20–22° C.

The duration of flight to the point of complete exhaustion varied widely within a group of flies from the same culture, but there was no obvious relation with sex. The age of the fly affected the duration of flight as already described by Williams *et al.* (1943); the period of flight is shorter in the very young and very old flies; but the total duration in *D. melanogaster* is much greater than that recorded by these authors in the larger species *D. funebris*. The results were as follows:

Flies 18–20 hr. old: 60, 75, 89, 92, 115, 127, 130, 145, 160, 170, 185, 187, 195: average 133 min.

Flies 1 week old: 195, 195, 200, 230, 245, 250, 275, 315, 320, 320, 325, 330, 345, 345: average 278 min.

Flies 4 weeks old: 50, 70, 75, 75, 80, 80, 80, 80, 85, 85, 95, 100, 105, 110, 115, 115, 120, 125, 130, 135, 180: average 100 min.

The corresponding values obtained by Williams *et al.* (1943) in *D. funebris* were 26, 110 and 20 min.

Flight behaviour of the exhausted flies. As they approach exhaustion the flies come to rest with increasing frequency, until they have to be restarted every minute or so. Then it can be noticed that instead of folding the wings the instant they cease to vibrate, the insect may stop with the wings extended as though it were still trying to fly. Finally, a point is reached at which, on withdrawing the cork, the wings are extended, make a few feeble beats and then stop. Exhaustion is then regarded as complete.

After a short rest, however, the exhausted insect may be induced to fly once more. It soon comes to rest, but will fly again for a shorter period still, usually only a few seconds, and the process can be repeated with gradually shortening periods of flight until exhaustion is once more complete. Until exhaustion is complete it is usually possible to restart flight within 1 sec. of the arrest. The duration of each short spell of flight can be timed, and the total duration of flight before exhaustion recorded. It then becomes evident that the total duration of flight of which the exhausted insect is capable is determined by the length of the preceding period of rest. The following is an example:

Duration of rest in min.	Successive flights in sec.	Total duration of flight in sec.
60	75, 4, 9, 5, 3	96
20	32, 10, 9, 5, 3, 3, 3	65
1	12	12
1	11	11
10	24, 5, 4, 3	36
20	33, 5, 3, 3	44
1	10, 3, 2	15
15	27	27

Naturally there is much individual variation. Fig. 4 summarizes the results obtained by these methods in a group of five flies of mixed sexes, some 1 week, some 4 weeks old. In spite of the variability in the results the increase in the duration of flight with the duration of rest is evident.

Changes in the glycogen reserves during flight. During the first hour of continuous flight there is a general consumption of glycogen in all the reserves. This almost disappears in visible form from the spaces between the muscle bundles. It is reduced in concentration throughout the vacuoles of the fat body—rather more strikingly in the thorax than in the abdomen. It is also reduced in the halteres. Everywhere the reduction is one of concentration in existing vacuoles rather than the complete discharge of certain deposits.

In flies which have reached the stage of exhaustion, or have made one or two post-exhaustion flights, there is a difference between old and young insects. In young flies, 5–7 days old, the exhaustion of glycogen in the fat body is much more complete; many of the cells are entirely devoid of glycogen, some have small deposits, and here

and there are groups of cells with a considerable amount of glycogen still present (Fig. 3C). The halteres usually still show pale staining deposits. In old flies, 4 weeks old, there is much more glycogen remaining in the fat body; and the halteres, although their glycogen is being drawn upon, often still contain deeply staining deposits.

Evidently two factors are involved in the reduced duration of flight in the old insects: the total reserves of glycogen are smaller, and their mobilization is less efficient (cf. Williams *et al.* 1943). This is reflected in the more frequent stimuli needed to keep the older insects in flight, long before exhaustion is complete.

In both groups of flies the oöcytes in the female still contain large amounts of untouched glycogen. But besides this, the residual glycogen noted by Williams

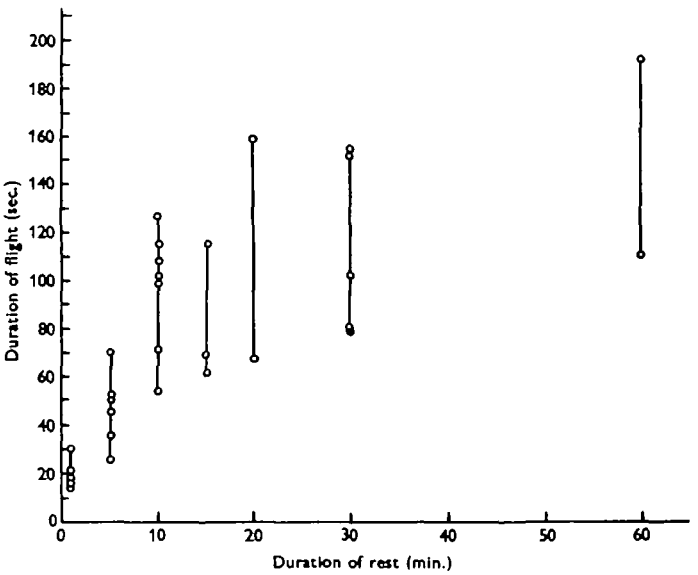

Fig. 4. Total duration of flight in exhausted *Drosophila* after varying periods of rest. Data from five flies of mixed age and sex.

et al. (1943) in the exhausted flies comprises persistent reserves in fat body and halteres. 'Exhaustion' supervenes when the rate of consumption of glycogen during flight exceeds the rate of mobilization.

Changes in the fat reserves during flight. In the normal insect, 5 days old, the droplets of fat in the fat body lie mostly around the nucleus at the centre of the cell with the distended glycogen vacuoles around (Fig. 3A, A'). In insects flown to exhaustion the cells are greatly shrunken, but the fat shows no apparent reduction—indeed, it often gives the impression of being increased because the fat droplets now fill the cell completely and in some places have run together to form larger droplets (Fig. 3C').

Thus, whereas in starvation, glycogen and fat are consumed at parallel rates, during flight the glycogen is used up, leaving the cell laden with fat.

UTILIZATION OF SUBSTANCES FOR FLIGHT AFTER EXHAUSTION

It was suggested by Chadwick (1947) that the exhausted fly might prove good material for comparing the utilization of different substances as sources of energy for flight. The fly in this state usually refuses to drink plain water. If water is taken, it makes no difference to the capacity for flight. Desiccation is not the cause of exhaustion. If the exhausted insect is given a suitable sugar the ability to fly is restored in varying degree.

Method. The sugar is offered at the tip of a waxed capillary pipette of the type previously described (Wigglesworth, 1937). The concentration used, for all the substances tested, has been 10% w/v (that is, rather less than M/2 in the case of hexose monosaccharides). Substances which appear to taste sweet for the fly are taken up greedily as soon as they touch the anterior tarsi or the proboscis; about 0·1 mm.^{3} is absorbed within 30 sec. Other substances may require 1–2 min. for ingestion; and a few of the substances tried are not taken up unless 10% sorbose (which is not utilized by the fly, but apparently tastes sweet) is added to the mixture.

It has been found convenient to exhaust the insects on one day, to keep them overnight without feeding, to exhaust them again next morning and to record the total duration of flight following a rest of 1 min. They are then given the test substance, and any change in the flight behaviour, notably any change in the total duration of flight after a rest of 1 min., is observed.

Results. Before summarizing the results obtained with all the substances tested, five experiments will be recorded in full in order to illustrate the types of response.

Exp. 1 shows that glucose becomes available, in sufficient quantity to maintain uninterrupted flight, within 30–45 sec. after ingestion.

Exp. 1. Male fly 6 days old flown to exhaustion. Duration of flight after 1 min. rest: 7, 5, 3 (= 15 sec.). Given just over 0·1 mm.^{3} of 10% glucose during 30 sec. immediately after flight. Flight was started at the end of this time and was then continuous for at least half an hour.

Exp. 2 shows that lactose has no effect on the duration of flight following a rest period of 1 min., at least up to 5½ hr. after ingestion; but at the end of this time continuous flight is restored within 60–90 sec. after the ingestion of maltose is begun.

Exp. 2. Female fly 6 days old flown to exhaustion. Duration of flight after 1 min. rest: 10, 3, 2, 2 (= 17 sec.). 1 min. rest: 8, 3, 3 (= 14 sec.).

Given 10% lactose; more than 0·1 mm.^{3} taken.

Immediately after feeding: 5, 4, 3, 2 (= 14 sec.). 1 min. rest: 6, 4, 3 (= 13 sec.). 5 min. rest: 19, 5, 4 (= 28 sec.). 5 min. rest: 21, 4, 3 (= 28 sec.). 30 min. rest: 34, 5, 3, 3, 2 (= 47 sec.). 1 min. rest: 15, 4 (= 19 sec.). 1 min. rest: 15, 4 (= 19 sec.). 1 min. rest: 10, 3 (= 13 sec.). 1 min. rest: 12, 4 (= 16 sec.). 1 min. rest: 10, 3 (= 13 sec.). 2 hr. 10 min. rest: 60, 5, 4, 3, 4, 3, 3, 2, 2 (= 86 sec.). 1 min. rest: 11, 4 (= 15 sec.). 1 min. rest: 9, 4, 2 (= 15 sec.). 1 min. rest: 9, 3, 2 (= 14 sec.). 2 hr. 15 min. rest: 45, 5, 4, 3 (= 57 sec.). 1 min. rest: 9, 4, 3 (= 16 sec.). 1 min. rest: 9, 3, 3 (= 15 sec.).

Given 10% maltose; about 0·1 mm.^{3} taken in 30 sec.

Immediately after feeding: 11, 5, 6, 9, 9, 7; flight became continuous.

Exp. 3 shows that xylose will not support continuous flight; but after 2 hr. or less it is being assimilated and made available rapidly enough to support brief flights lasting 6–7 sec. and repeated indefinitely.

Exp. 3. Male fly 6 days old flown to exhaustion. Duration of flight after 1 min. rest: 7, 2 (=9 sec.). 1 min. rest: 7, 2, 2 (=11 sec.).

Given 10% xylose; about 0·15 mm.^{3} taken.

Immediately after feeding: 8, 3, 2 (=13 sec.). 1 min. rest: 7, 3, 2 (=12 sec.). 1 min. rest: 8 (=8 sec.). 1 min. rest: 10, 4 (=14 sec.). 1 min. rest: 9, 3 (=12 sec.). 1 min. rest: 7, 5 (=12 sec.). 15 min. rest: 28, 9, 5 (=42 sec.). 2 hr. 45 min. rest: 160, 10, 8, 5, 6, 6, 8, 7, 5, 6, 6, 7..., etc. 1 min. rest: 32, 7, 7, 6, 7, 7, 6, 6,... etc. 1 min rest: 11, 12, 10, 7, 7, 6, 7, 7, 6..., etc.

Exp. 4 shows that mannitol will not support continuous flight, nor maintain repeated short flights, but the total duration of flight after a rest period of 1 min. may be more than trebled.

Exp. 4. Female fly 1 week old flown to exhaustion.

Duration of flight after 1 min. rest: 12 (=12 sec.). 1 min. rest: 10 (=10 sec.). 1 min. rest: 9 (=9 sec.).

Given 10% mannitol; about 0·2 mm.^{3} absorbed during 2 min.

Immediately after feeding: 18 (=18 sec.). 1 min. rest: 18 (=18 sec.). 1 min. rest: 17 (=17 sec.). 1 min. rest: 14, 5 (=19 sec.). 1 min. rest: 17, 4 (=21 sec.). 1 min. rest: 15, 2 (=17 sec.). 1 min. rest: 32 (=32 sec.). 1 min. rest: 34 (=34 sec.). 1 min. rest: 25 (=25 sec.).

2 hr. later: made about 5 min. continuous flight. Then some long flights before exhaustion. 1 min. rest: 35, 11, 7, 3 (=56 sec.). 1 min. rest: 36, 3, 3 (=42 sec.). 1 min. rest: 32, 3, 2, 2 (=39 sec.).

Exp. 5 shows that glycine leads to a *shortening* of the duration of flight after a rest period of 1 min.

Exp. 5. Male fly 1 week old, flown to exhaustion.

Duration of flight after 1 min. rest: 6, 3, 2 (=11 sec.). 1 min. rest: 7, 3 (=10 sec.). 1 min. rest: 5, 3, 2 (=10 sec.).

Given 10% glycine; about 0·15 mm.^{3} taken in 45 sec.

Immediately after feeding: 8, 2 (=10 sec.). 1 min. rest: 5, 2 (=7 sec.). 1 min. rest: 4, 2 (=6 sec.). 1 min. rest: 3, 2 (=5 sec.). 1 min. rest: 2, 2 (=4 sec.). 1 min. rest: 3, 2 (=5 sec.). 1 min. rest: unable to fly in spite of making many apparent attempts. 1 min. rest: 2, 2 (=4 sec.). 1 min. rest: 4 (=4 sec.). 1 min. rest: 2 (=2 sec.). 1 min. rest: 2, 2 (=4 sec.).

Summary of results. All the substances studied fell within one or other of five categories. No doubt there are differences between the substances in a given category; but with a few exceptions (notably in the group giving continuous flight) they are obscured by individual variation among the insects. All substances were tested at the single concentration of 10%, from four to eight experiments being made with each. They are grouped as follows:

(i) Substances which will support continuous flight (Exps. 1 and 2): *glucose, fructose, mannose, sucrose, maltose, trehalose.*

Glucose is the most efficient; it will restore continuous flight within 30–45 sec. of ingestion. Fructose required 2–3 min. Mannose also required several minutes. Sucrose, maltose and trehalose required from 1 to 1½ min.

(ii) Substances which will not support continuous flight but lead to repeated short flights of a few seconds which can apparently be continued indefinitely with a pause of a second or so between each (Exp. 3): *galactose, xylose, sorbitol, α-methyl-*D*-glucoside.*

(iii) Substances which lead only to an increase in the total duration of flight following a standard rest period of 1 min. (Exp. 4): *mannitol, glycerol, inositol.*

(iv) Substances which have no effect on the duration of flight (Exp. 2): *sorbose, rhamnose, arabinose, lactose, cellobiose, dulcitol, ethyl alcohol,* acetic acid** (as sodium salt), *lactic acid** (free and as sodium lactate), *β-hydroxybutyric acid** (as sodium salt).

(v) Substances which lead to a decrease in the total duration of flight following a standard rest period of 1 min. (Exp. 5): *glycine, alanine.*

THE RATE OF CONSUMPTION OF CARBOHYDRATE DURING FLIGHT

By feeding exhausted flies with a measured volume of glucose solution of known concentration, it has been possible to estimate directly the rate of consumption of carbohydrate during flight.

A fine wax-lined capillary pipette (Wigglesworth, 1937) has been used. It is graduated as follows. A column of mercury occupying about 5 mm. is introduced and its extremities marked. It is then discharged, mounted in Canada balsam, its diameter measured with a micrometer eyepiece and its volume calculated. A drop of water of this volume, that is, a drop which exactly fills the space between the two marks, is then introduced into the pipette and moved along so that the pipette is graduated into a series of segments of equal volume though of slightly varying length. Each segment is then bisected to give the unit actually employed—occupying a length of 2·5–3 mm. For measurement, each unit is divided into tenths by eye. In the pipette used, the unit had a volume of 0·024 mm.[3]

Flies 24 hr. old were used. These were mounted and exhausted, kept overnight, and re-exhausted next morning so that even after a prolonged rest they were incapable of more than a few seconds flight. They were then given a measured volume of 5 or 20% glucose and kept flying continuously until completely exhausted again. In most cases the end-point was quite sharp, that is, within about 1 min. The results are summarized in Table 1.

The duration of flight for a given weight of glucose varies considerably, but it tends to be greater in the smaller insects. In the culture used, flies of mixed sex 1–2 days old had an average weight of 0·57 mg. As shown in Table 1 the mean duration of flight on 1 μg. of glucose is 6·3 min. (3·6–10·0).

It is interesting to compare this value with those deduced by Chadwick & Gilmour (1940) and by Williams *et al.* (1943). Chadwick & Gilmour used the much larger *D. repleta* (average weight 3·5 mg.). The average consumption of glycogen

* Ingested by the fly only if 10% or so of sorbose was added.

during a flight of 1 hr. (calculated from the oxygen uptake) is given as 0·1 mg. 1 μg. of carbohydrate will therefore last this larger fly 0·6 min. only.

Williams *et al.* used *D. funebris*. This had a glycogen content of 4·88% of the live weight, which was reduced to 1·30% of the live weight after 90 min. flight. If it be assumed that *D. melanogaster* (although a much smaller species) contains the same average percentage glycogen, and uses this at the same percentage rate, it can be calculated that it would consume 21·5 μg. in 90 min. So that 1 μg. would last 4·3 min.—a value which falls within the range observed above: 3·6–10·0 min.*

Finally, it is of some interest to form an estimate of the value of the glycogen contained in the knobs of the halteres as a reserve for flight. These knobs may be regarded as spheres about 0·11 mm. in diameter and with a volume therefore of 0·000695 mm.^{3} Assuming, as a very rough approximation, that the sphere has a specific gravity of 1 and may contain 50% of glycogen, then the carbohydrate carried in the two halteres would amount to 1·4 μg. and would provide for about 4½ min. flight—say one-sixtieth of the total.*

Table 1

Insect used	Concentration of glucose (%)	Volume taken (mm.^{3})	Glucose taken (μg.)	Total duration of flight (min.)	Duration of flight in minutes per μg. glucose
A. Large male	20	0·204	41	152	3·6
B. Very small female	20	0·217	44	290	6·6
C. Average male	20	0·077	15	83	5·5
D. Rather small female	20	0·109	22	94	4·3
E. Average male	20	0·082	16	90	5·6
F. Small female	5	0·065	3	30	10·0
G. Average male	5	0·075	4	21	5·2
H. Small female	5	0·067	3	26	8·7
J. Large female	5	0·108	5	27	5·4
K. Average female	5	0·072	3·5	25	7·1
L. Average female	5	0·088	4	28	7·0

DISCUSSION

During starvation *Drosophila* utilizes both fats and carbohydrates. The fasting fly expends its energy in running around its container. There seems little doubt that fat and glycogen are both being used for muscular activity. It has long been known that grasshoppers lay down large stores of fat before making migratory flights; the fat content of the beet leafhopper (*Eutettix*) may be used as a measure of the distance over which it has flown (Fulton & Romney, 1940); and recently Krogh & Weis-Fogh (1948) have shown that the respiratory quotient of the desert locust *Schistocerca* during sustained flight equals 0·74–0·75. Yet the work of Chadwick & Gilmour (1940) and Chadwick (1947) has clearly proved, and the present observations show nothing to the contrary, that *Drosophila* during flight consumes only carbohydrate.

The difference is probably one of speed of metabolism. Glycogen, which is stored in large amounts in *Drosophila*, is the most readily available source of energy.

* For the purpose of these approximations it has been assumed that glycogen has the same calorific value as glucose.

So long as it can be mobilized with sufficient rapidity to meet the high metabolic demands of the indirect muscles, flight is continuous. 'Exhaustion' supervenes when the reserve deposits are reduced to such a level that the rate of mobilization can no longer keep pace with the consumption. It is suggested that the conversion of fats into a form which will provide an immediate source of energy for muscular contraction in the fibrillar muscles cannot take place with the necessary speed and therefore the fly appears unable to use fats for flight.

The same argument may be applied to the substances given by mouth to the exhausted fly. It is highly probable that some of the substances in groups (iv) and (v), such as lactic acid, ethyl alcohol, glycine and alanine, can serve as sources of muscular energy. The two amino-acids are certainly efficient sources of glycogen in insects (Wigglesworth, 1942) as in other animals. The most likely explanation of their apparent failure to improve the powers of flight in the exhausted fly is that the chemical transformations necessary take too long a time.

In the case of substances in group (iii), like glycerol or mannitol, the speed of transformation is sufficient to increase the total duration of flight after a rest of 1 min., but not sufficient to support continuous flight. Substances of group (ii), like galactose or xylose, are transformed rapidly enough to give repeated brief flights but not quite rapidly enough to give uninterrupted flight. And among the group (i) substances it requires a little longer for fructose and mannose to become available in suitable form and adequate amount than it does for glucose. The delay amounting to half a minute or so, in the case of sucrose, maltose and trehalose is doubtless due to the time taken to effect hydrolysis, and the failure in the case of lactose and cellobiose results no doubt from the absence of the necessary enzymes.

The effect of glycine and alanine (group (v)) in actually shortening the duration of flight after a rest of 1 min. is interesting. Perhaps it signifies a diversion of carbohydrate, which would have been moved to the muscles, into some new channel. One thinks of the 'specific dynamic action' exerted by these amino-acids.

In general, the results agree very well with those obtained recently by Hassett (1948), who studied the effect of a long list of substances on the survival time of *D. melanogaster*. The numbers in brackets in the following lists represent the mean time of survival in days, as recorded by Hassett, in flies fed solely upon the substances used in the present study.

Group (i): glucose (16), fructose (18), mannose (14), sucrose (24), maltose (17), trehalose (21).

Group (ii): galactose (9), xylose (7), sorbitol (5), α-methyl-D-glucoside (6).

Group (iii): mannitol (6), glycerol (14), inositol (6).

Group (iv): water alone (2), sorbose (2), rhamnose (2), arabinose (2), lactose (2), cellobiose (2), dulcitol (2), ethyl alcohol (2), acetic acid (3), lactic acid (5).

Group (v): glycine (3), alanine (2).

In Hassett's experiments the presence or absence of digestive enzymes, the ease of metabolism, and the acceptability to the fly must all have been involved.

The results on *Drosophila* agree well also with those obtained in the mosquito larva *Aedes* (Wigglesworth, 1942), in which the efficiency of substances as sources

of glycogen was compared. A curious exception is trehalose, which, oddly enough, was reported as being no better than galactose. Glycine and alanine were good glycogen formers.

The presence of dense deposits of glycogen, and the virtual absence of fat, in the cells of the haltere knob, is of little significance for the supply of energy. Perhaps the function of this glycogen is mechanical. Glycogen has a specific gravity of about 1·5. Its presence will increase the mass of the knob and add to its inertia. As a result it may improve slightly the efficiency of the haltere as a gyroscopic sense organ (Pringle, 1948).

SUMMARY

The chief reserve substance in *Drosophila* is glycogen. This forms dense deposits in the cells of the fat body and the haltere knobs. It is distributed throughout the indirect flight muscles as minute granules in the meshwork of sarcoplasm that fills the space between the fibrils and the sarcosomes. Somewhat larger masses lie along the surface of the fibre bundles and around their insertions. There is a large deposit in the proventriculus and small amounts in the cells of the mid-gut. The visible deposits of fat are much smaller and are confined to the fat body and the mid-gut cells.

During starvation, glycogen and fat are consumed concurrently. At the time of death (2–3 days) glycogen has disappeared completely, save in the thoracic muscles; only minute droplets of fat remain in the fat body.

In the insect which has flown to exhaustion (4–5 hr. in the mature fly) there is no apparent reduction in the stored fat. Glycogen is greatly reduced in all the deposits, but has disappeared completely only in the flight muscles and the proventriculus.

'Exhaustion' of the flying insect supervenes when the glycogen can no longer be mobilized rapidly enough to meet the metabolic demands of the flight muscles. Flight can be resumed for a brief period after the exhausted fly has rested; and the duration of flight increases with the duration of rest.

By observing the duration of flight after giving a measured quantity of sugar to the exhausted insect it is shown that 1 μg. of glucose will maintain *D. melanogaster* in flight for an average of 6·3 min.

The efficiency of substances as sources of energy for flight has been compared by giving them to the exhausted fly. Glucose will restore the capacity for continuous flight within 30–45 sec. of the commencement of feeding. Fructose, maltose, sucrose, etc., require a little longer. Galactose, xylose, etc., will allow repeated brief flights but will not support uninterrupted flight. Mannitol, glycerol, etc., merely increase the duration of flight after a standard period of rest. Lactose, sorbose, etc., have no effect. Glycine and alanine actually diminish the capacity for flight.

It is suggested that the apparent failure of fats, etc., to support flight in *Drosophila* is due to the comparatively slow rate of their metabolism.

It is suggested that the deposits of glycogen in the haltere knob may serve to increase the inertia of the haltere and so its efficiency as a gyroscopic sense organ.

I am indebted to Mrs A. Whittingham for technical assistance of many kinds and to Prof. A. Krogh and Dr T. Weis-Fogh for permission to quote their unpublished work.

REFERENCES

CHADWICK, L. E. (1947). *Biol. Bull. Woods Hole*, **93**, 229–39.
CHADWICK, L. E. & GILMOUR, D. (1940). *Physiol. Zoöl.* **13**, 398–410.
FULTON, R. A. & ROMNEY, V. E. (1940). *J. Agric. Res.* **61**, 737–43.
HASSETT, C. C. (1948). *Biol. Bull. Woods Hole*, **95**, 114–23.
HOLLICK, F. S. J. (1940). *Philos. Trans.* B, **230**, 357–90.
KROGH, A. & WEIS-FOGH, T. (1948). Unpublished work.
PRINGLE, J. W. S. (1948). *Philos. Trans.* B, **233**, 347–84.
SIANG-HSU, W. (1947). *J. Morph.* **8**, 161–84.
WIGGLESWORTH, V. B. (1930). *Quart. J. Micr. Sci.* **73**, 593–616.
WIGGLESWORTH, V. B. (1937). *Biochem. J.* **31**, 1719–22.
WIGGLESWORTH, V. B. (1942). *J. Exp. Biol.* **19**, 56–7.
WILLIAMS, C. M., BARNESS, L. A. & SAWYER, W. H. (1943). *Biol. Bull. Woods Hole*, **84**, 263–72.