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Metabolism of the Bloodmeal in Tsetse Flies

(a Review)

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Contents

1. Introduction: The metabolic problem

The tsetse fly is an obligatory bloodsucker inhabiting environments which are often extremely arid, and as such it faces a metabolic problem. The large amount of nitrogen which its food contains must, in the interests of water conservation, be disposed of largely as uric acid. This requirement would not be easy to reconcile with a carbohydrate-based metabolism, as the main uric acid precursor, glycine, and its two closely related 3-carbon amino acids, serine and alanine, are the very amino acids which could most easily be converted to 3-carbon intermediaries of the gluconeogenic pathway. Other amino acids, which degrade primarily to 4-carbon or to 2-carbon fragments (see Fig. 1) could not economically be converted to 3-carbon fragments, especially in view of the irreversibility of the reaction which converts pyruvate to acetyl CoA by oxidative decarboxylation. For this reason the standard pattern of dipteran metabolism, which involves the use of carbohydrate as the main food reserve, could not easily be retained. On the other hand, the ready availability of 2-carbon acetyl CoA, which is an effective precursor for the synthesis of fatty acid, would make triglyceride a suitable storage form, and it has long been known that fat constitutes the main

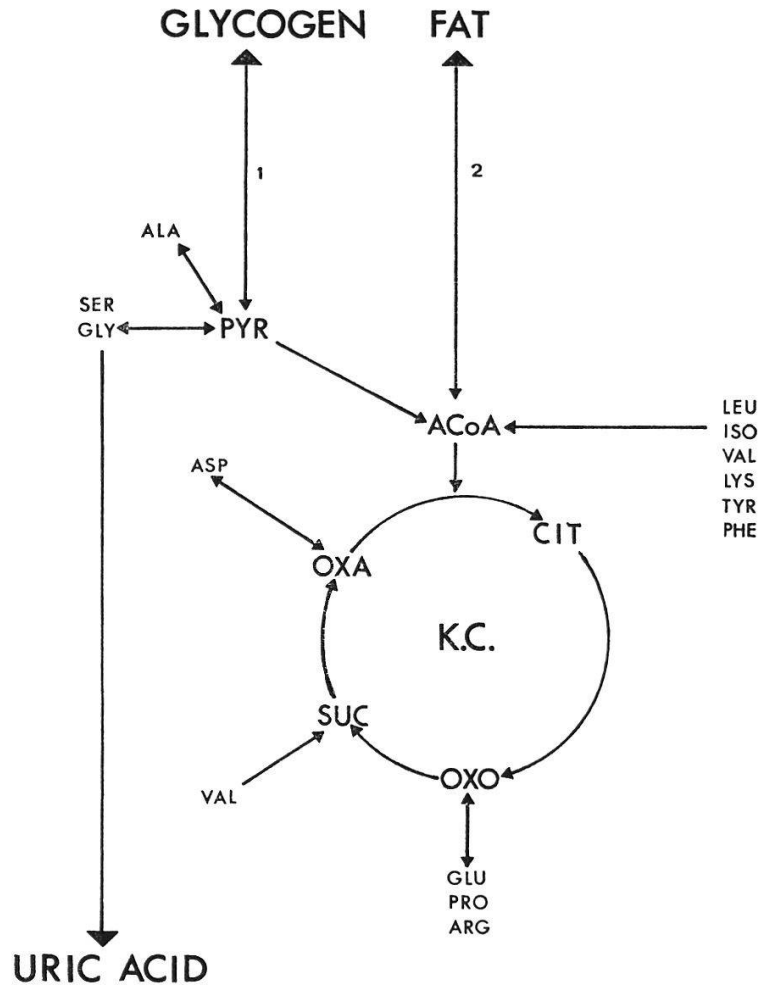

Fig. 1. The relationship of major amino acids to the Krebs' cycle (K.C.), and to the glycogenic (1) and lipogenic (2) pathways. In this, as in all subsequent figures, substrates will be denoted by the first three letters of their name. ACoA = acetyl Coenzyme A.

food reserve of tsetse flies (e.g. JACK, 1939). But this would raise another difficulty, because it is well known that fat cannot be mobilised quickly to meet the needs of flight metabolism in insects, and species which use fat as their main food reserve, such as the locust, have to use carbohydrates to sustain initial phases of flight (WEIS-FOGH, 1952). In view of the proximity of many amino acids to the energy-yielding Krebs' cycle (see Fig. 1) these substances could clearly substitute for carbohydrate to provide energy for initial stages of flight, but their use for this purpose would involve a rapid release of ammonia, and because of the general toxicity of this substance this might have dangerous consequences.

In view of these general considerations it is clear that the adoption of a blood-sucking mode of life by a flying insect, such as the tsetse, would pose a substantial metabolic challenge, and it has been our aim to establish the mechanisms by which this challenge has been met. Our understanding of the processes involved is by no means complete, but in broad outline the situation is clear, and the stage has been reached when a preliminary review of progress may be useful.

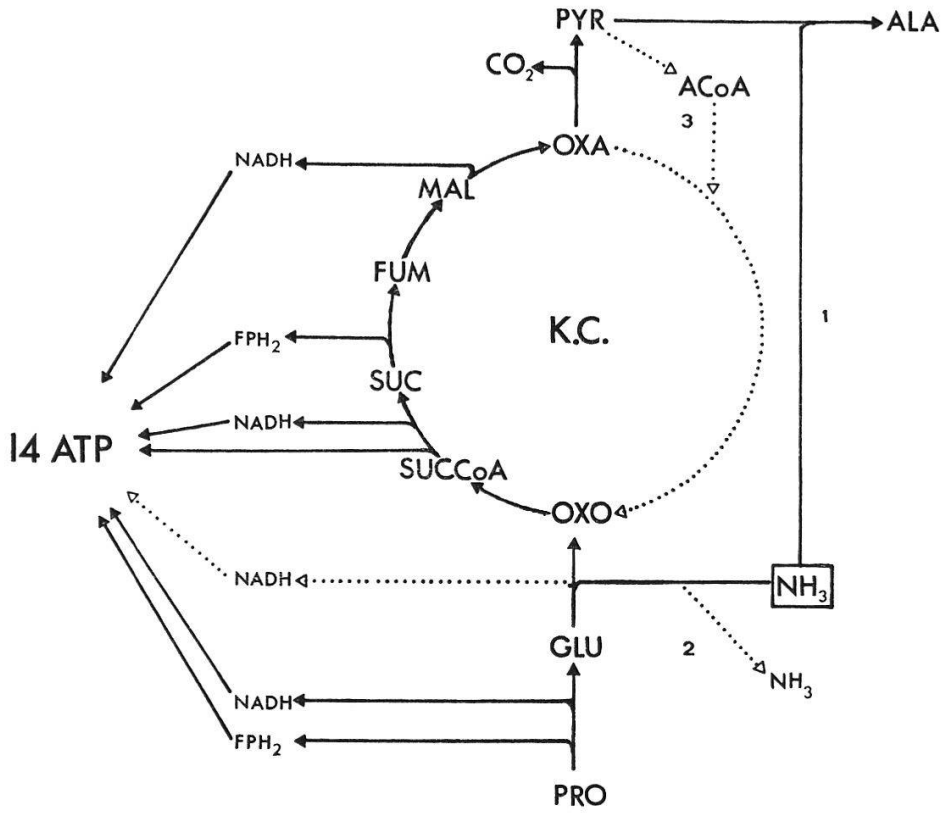

Fig. 2. The partial oxidation of proline in flight muscles of the tsetse fly. The main pathway involves the transfer of an amino group from glutamate to pyruvate (1), but during early stages of flight glutamic dehydrogenase is active (2), providing for the total oxidation of proline through pyruvate and acetyl CoA (3). For further explanation see text. NADH = reduced nicotine adenine dinucleotide; FP_{H_2}= reduced flavoprotein; K.C. = Krebs' cycle. (From data in BURSELL, 1963 & 1967; HARGROVE, 1973.)

2. The role of proline in metabolism

Early work indicated that proline plays an important part in the flight metabolism of the tsetse (BURSELL, 1963), and subsequent investigations have served to emphasize the central importance of this amino, or rather imino, acid as a substrate for oxidative metabolism, and as a source of energy for muscle during rest as well as during flight.

(a) The pathway of oxidation

The metabolic pathway by which proline is oxidized during flight (see Fig. 2) involves an initial oxidation of proline to glutamate, which is transaminated with pyruvate as an aminoacceptor, to give alanine and oxoglutarate. The keto-acid is then oxidized by normal operation of the Krebs' cycle to oxaloacetate, which decarboxylates to replace the pyruvate used in the initial transamination. Recent work has shown that

during the earliest phases of flight part of the oxoglutarate arises by oxidative deamination of glutamate, leading to a loss of amino-nitrogen (HARGROVE, 1973) and presumably to the appearance of free ammonia as indicated by the dotted lines in Fig. 2, but the indications are that this pathway becomes unimportant during later phases of flight (see also section 1d below).

(b) The energy yield

The overall reaction for the partial oxidation of proline to alanine is

$$\text{proline} + 2^1/_2\,O_2 + 14\ \text{ADP} \rightarrow \text{alanine} + H_2O + 2\,CO_2 + 14\ \text{ATP} \qquad (1)$$

and it is interesting to note the high yield of energy provided by this pathway in terms of the quantity of ATP which is made available per unit mass of material lost in oxidation. In the oxidation of one mole of proline (molecular weight = 115) to one of alanine (molecular weight = 88) 27 g of material is lost, and this provides 14 moles of ATP, giving 0.52 moles ATP/g of material combusted. The corresponding value for glucose is only 0.18, while fat yields 0.69. It would appear that in its partial oxidation proline combines the high energy yield of a lipid with the ready mobilisability of a carbohydrate, and that the excessive build-up of ammonia which would normally be involved in the oxidation of an amino acid is avoided by use of a coupled transamination.

During the earliest phase of flight, when oxidative deamination is active, a portion of the pyruvate which arises as a partial oxidation product will not be required to serve as an amino acceptor for the transaminase, and this pyruvate would therefore become available, through acetyl CoA, for further oxidative manipulation of the Krebs' cycle (see dotted lines in Fig. 2), giving total oxidation of proline according to the following reaction

$$\text{proline} + 5^1/_2\,O_2 + 32\ \text{ADP} \rightarrow 5\,CO_2 + 3\,H_2O + NH_3 + 32\ \text{ATP} \qquad (2)$$

Here the yield of ATP per mole of proline is substantially increased, from 14 to 32 moles, but in this process the loss of material is total at 115 g per mole of proline giving 0.28 moles of ATP per g; this is a much lower value than that for partial oxidation, but still substantially greater than that which characterises the total oxidation of carbohydrate.

(c) The enzymatic machinery of proline oxidation

The properties of sarcosomes isolated from the flight muscles of the tsetse have been found to reflect the special metabolism of this insect in a number of ways, as shown in Table 1. Section A shows that proline is

Table 1. The oxygen consumption of tsetse flies during flight, as estimated from rates of proline consumption, compared with the activity of some of the enzyme systems involved. The activity of systems associated with carbohydrate and lipid metabolism have been included for comparison.

System		Activity	Reference
A. *Oxygen consumption*		μl O_2/min/g WW	
Proline "oxidase"	*in vivo*	4,000	1
Proline "oxidase"	*in vitro*	5,000	2
α-glycerophosphate "oxidase"	*in vitro*	500	2
Pyruvate "oxidase"	*in vitro*	50	2
Palmitoyl carnitine "oxidase"	*in vitro*	30	2
B. *Substrate consumption*		μmoles/min/g WW	
Proline "oxidase"	*in vivo*	70	1
Alanine-oxoglutarate amino-transferase	*in vitro*	400	3
Proline dehydrogenase	*in vitro*	60	3
Glutamic dehydrogenase	*in vitro*	50	4
C. *Product formation*			
Glycolysis (pyruvate from fructose-6-phosphate)	*in vitro*	0.1	5

The term "oxidase" is used to denote the total system involved in the oxidation of the substrate specified.

All values have been expressed in terms of flight musculature (i.e. per g wet weight of thoracic muscle) for purposes of converting values reported in other terms, the wet weight of a male *G. morsitans* has been taken as 25 mg, of which 10 mg is constituted by flight musculature, and this contains 1 mg of mitochondrial protein and 0.95 nmoles of cytochrome *c* (from data in BURSELL, 1973; SLACK & BURSELL, 1972; HARGROVE, 1973). It has been assumed that proline is oxidised according to formula 1 of section 2b, so that 1 μmole of proline is equivalent to 5 μatoms, or 56 μl, of oxygen.

1. BURSELL, 1963; 2. BURSELL & SLACK, 1970 and in preparation; 3. CRABTREE & NEWSHOLME, 1970; 4. BURSELL, in preparation; 5. NORDEN & PATERSON, 1970.

oxidised very rapidly by isolated mitochondria, at rates adequate to account for the oxygen consumption of flies during the early stages of flight (as calculated from the rate of disappearance of proline). The systems involved in the oxidation of carbohydrates and lipids are by comparison extremely poorly developed, and it is clear that they can play little part in the provision of energy for flight.

Apart from enzymes of the Krebs' cycle, there are three enzymes that are specifically involved with flight metabolism in the tsetse fly – proline dehydrogenase, glutamate-pyruvate aminotransferase and glutamic dehydrogenase. Section B of Table 1 shows that the activity of the aminotransferase in homogenates of tsetse flight muscle is more than adequate to account for the observed rate of proline consumption. The specific activities of proline dehydrogenase and of mitochondrial glu-

tamic dehydrogenase are substantially lower, but at the right general level to account for the observed rates of oxidation.

The enzyme responsible for the formation of pyruvate remains to be identified. Oxaloacetic decarboxylase has been shown to be highly active in homogenates of tsetse flight muscle (BURSELL, 1965a), but most of the activity is associated with the soluble fraction, and it is clear that it can play no part in the intramitochondrial events under discussion.

The minor role played by carbohydrates in the flight of the tsetse is confirmed by the low activity of the glycolytic system (Section C); pyruvate would be made available at less than 2 % of the rate at which proline is oxidised.

It is of interest to compare the rate of oxygen consumption during flight with the values obtained by RAJAGOPAL and BURSELL (1966) for the resting fly. At 25° the average rate was 22 μl/h/fly, which would be equivalent to 15 μl/min/g wet weight of insect. This compares with a value of 4,000 μl/min/g wet weight of muscle (see Table 1, A), or 1,600 μl/min/g wet weight of insect, in other words the rate of metabolism increases over a hundred times during flight. The increase is within the range of values reported by CHADWICK (1953) although high compared with most insect species; this is to be expected in view of the fact that flight musculature constitutes a higher proportion of total body weight in tsetse flies than in most other insects (HARGROVE, 1973).

(d) Regulatory aspects

The transition from the resting to the active state in insect flight muscle has been studied intensively during recent years (see review by SACKTOR, 1970) and it is thought that 3 factors are primarily involved in the activation of the metabolic machinery during transitions from the resting to the active state: the increase in calcium concentration which follows release of calcium from the sarcoplasmic reticulum, and the increase in the concentration of ADP, and of inorganic phosphate, associated with the hydrolysis of ATP by the contractile mechanism.

In the tsetse the last two factors appear to be of primary importance; calcium is not required for maximal rates of proline oxidation in isolated sarcosomes, and none of the individual enzymes involved has a calcium requirement. On the other hand, the oxidation of proline by isolated sarcosomes is extremely tightly coupled, and occurs at negligible rates in the absence of exogenous ADP and phosphate (BURSELL & SLACK, 1969); and several of the enzymes involved have been shown to be activated by low concentrations of ADP: (NORDEN & VENTURAS, 1972) for proline dehydrogenase; BURSELL (in preparation) for glutamic dehydrogenase.

The switch from deamination to transamination during early stages of flight may be accounted for on the basis of product inhibition. Ammonia has been shown to act as a powerful competitive inhibitor of the oxidative deamination of glutamate, and total inhibition of the *in vitro* system occurs at a concentration of 25 mM. The amount required to bring the concentration of ammonia in body fluids to this level, assuming that there is no loss from the system, would be produced during the first minute of flight. The oxidative performance of sarcosomes is not affected by this concentration of ammonia if α-glycerophosphate is used as a substrate, indicating that the operation of the hydrogen transport system is unimpaired, and it must be assumed that other physiological processes are tolerant of transient exposure to this level of ammonia.

(e) Alternative substrates for flight metabolism

Accepting that proline may be regarded as the main substrate for flight metabolism the question would still remain whether the oxidation of substrates other than proline could contribute substantially to the total output of energy. In other insects it has been found that energy for early phases of flight derives from the oxidation of carbohydrate reserves, and deposits of glycogen, which might serve as a source of flight substrate, have been shown to occur in flight muscles of the tsetse (D'COSTA et al., 1973). The quantity involved is small compared with insects that use carbohydrates as a source of energy for flight, amounting to little more than 100 μg per fly, but at calculated rates of oxygen consumption it would be adequate to sustain flight for nearly two minutes. However, NORDEN and PATERSON (1969 and 1970) have shown that glycolytic enzymes, though present in homogenates of tsetse flight muscle, are much less active than those of other Diptera, and the same applies to mitochondrial systems involved in carbohydrate oxidation (see Table 1). It is clear that the glycogen present in tsetse muscle could not be metabolized quickly enough to supply energy to the active flight system at a significant rate. A contrary opinion has been expressed by NAYAR and HANDEL (1972) but these workers appear to have worked on the assumption that rates of respiration increase by a factor of about 7 during flight, whereas in fact it increases by a factor of more than 100.

In certain insects lipids have been shown to constitute an important substrate during later phases of flight (see review by SACKTOR, 1970), though this seems to apply mainly to species with relatively low wingbeat frequencies, like the locust and the cockroach, and has not been demonstrated in Diptera. However, since lipids constitute the main food reserve in tsetse flies, it seemed possible that they might prove to be of importance in flight metabolism.

The fact that isolated sarcosomes do not oxidize a normal lipid substrate of insect mitochondria such as palmitoyl carnitine (see Table 1, A) could not be considered conclusive in this context in view of the fact that insect mitochondria are notoriously prone to physiological deterioration during isolation. However, evidence became available from other sources which supported the idea that lipids were not the immediate substrate of oxidative metabolism. For one thing, the carnitine content of the thoracic musculature of tsetse flies is extremely low compared with that of insects which use lipids as a substrate for oxidative metabolism (Dr. E. A. Newsholme, personal communication). Secondly, it has been shown that the concentration of diglyceride, which is the normal transport form of lipids from fat stores to site of metabolism, is extremely low in the haemolymph of tsetse flies (K. C. BILLING, in preparation) and shows no increase during early stages of flight as would be expected if it constituted an important source of energy (cf. MAYER & CANDY, 1967; BEENAKKERS, 1973]. Taken together these findings strongly suggest that later stages of flight could not be sustained by direct oxidation of lipid.

(f) Reconstitution of the proline reserve

Earlier work had shown that during rest following flight in tsetse flies the proline reserves are reconstituted (BURSELL, 1963) and at this time a rapid incorporation of alanine-carbon and of bicarbonate-carbon into proline has been observed (BURSELL, unpublished; HARGROVE, 1973). This raised the possibility that in the resting fly alanine might serve as a precursor for the synthesis of proline in a process which involved a preliminary carboxylation. The situation was further investigated by MCCABE (1973), who showed that when radioactive fatty acids were administered to the resting fly, radioactivity appeared first in glutamic acid and proline, and it was only later that substantial quantities of radioactive carbon dioxide could be recovered. This finding provided unequivocal support for the view that fatty acid is not oxidized directly by the tsetse, and suggested that its oxidation involves a preliminary conversion to proline. It also served as the basis for a scheme of proline regeneration, accommodating all observations made on this aspect of metabolism, as illustrated in Fig. 3. The pathway involves a transamination of alanine with oxoglutarate as aminoacceptor, the carboxylation of the pyruvate so formed to give oxaloacetate, a condensation of this with 2-carbon fragments (acetyl CoA), arising from the β-oxidation of fatty acids, to produce citric acid which, by normal operation of the Krebs' cycle would provide oxoglutarate to replace that used in the initial transamination. The glutamate so formed would then be reduced to proline.

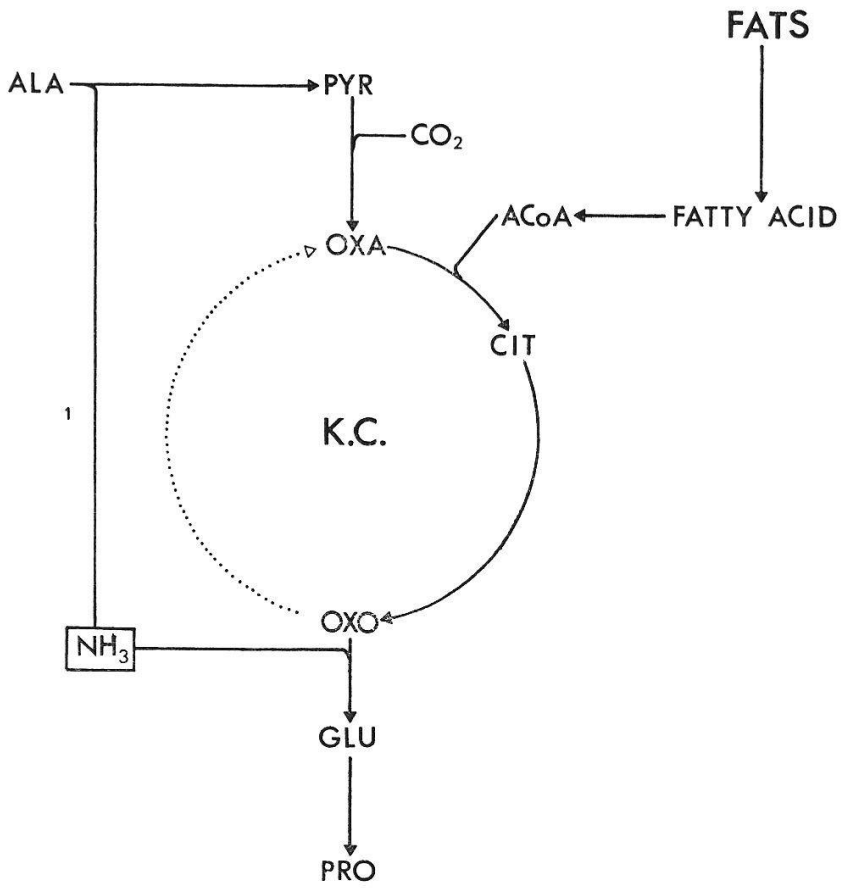

Fig. 3. A possible mechanism for the synthesis of proline, using alanine, bicarbonate and lipid carbon as precursors. The transfer of an amino group from alanine to oxoglutarate (1) is envisaged. For further explanation see text. K.C. = Krebs' cycle.

In this context it is important to note that the pathway for the oxidation of proline (Fig. 2) and for its synthesis (Fig. 3) both make use of the segment between oxoglutarate and proline, and both must involve the use of a mitochondrial transaminase, working in one case to promote the transfer of an amino group from glutamate to pyruvate, and in the other to promote the reverse transfer from alanine to oxoglutarate. It is obvious that these two opposite reactions could not proceed simultaneously at appreciable rates under the influence of a single catalytic system, and it was therefore necessary to postulate some form of compartmentalisation. The sarcosomes of flight muscle have been firmly identified as the site of proline oxidation, and the site of synthesis had to be sought elsewhere. In fact, the work of BURSELL (1966), of MCCABE (1973) and of HARGROVE (1973) all provides evidence for the existence of two distinct metabolic pools in respect of proline, alanine and glutamic acid, of which one can be identified with thoracic flight musculature, the other with abdominal fat body, or fat body generally. What must be envisaged, in the light of these con-

siderations, is that alanine, arising as a product of the partial oxidation of proline in the thorax, will be transported to the abdominal fat body where it is used as raw material for the resynthesis of proline. In this sense alanine could be regarded as a carrier of lipid carbon, and of the energy associated with lipid carbon, from fat body to flight muscle, and the system as a whole would provide a special mechanism for the oxidation of lipid, geared to the special proline-orientated metabolism of the tsetse fly.

It is not suggested that the system described would be capable of steady-state operation during flight, i.e. that proline could be reconstituted by the fat body as fast as it is being oxidized by the flight muscles. The gross disproportion in mass and in mitochondrial content between the two tissues would militate against this. Nevertheless, the evidence from radioactive tracer experiments (BURSELL, 1966; HARGROVE, 1973) suggest that the rate of reconstitution is by no means negligible in relation to flight consumption, and when it is considered that in fed flies alternative raw materials will be available for the synthesis of proline (see section 3d below), it is clear that the re-synthesis of proline during flight will materially increase the total amount of proline available, and hence delay the moment of proline exhaustion.

(g) Proline availability in relation to flight performance

The metabolic studies summarised above have indicated that proline may be the sole substrate of flight metabolism, and that it is incapable of being reconstituted during flight at rates commensurate with the rate of its utilisation. This suggests that the flight capacity of tsetse flies might be limited by the level of their proline reserve, and direct observations of flight activity seems to be in accord with this suggestion. The work of BRADY (1972) has shown that the average duration of bursts of activity, under conditions where the opportunity for flight is limited, is less than a minute. With the recent development, by CULLIS & HARGROVE (1972), of a flight mill suitable for use with tsetse flies, it has been possible to get estimates of flight duration under less confined conditions, and the average duration of flight for mature males of *Glossina morsitans* was just over two minutes (HARGROVE, 1973). This correlates well with the time at which average proline levels approach minimal values in the same species (BURSELL, 1963). The suggestion of limited flight duration also receives support from amino acid analyses of flies captured in the field (BURSELL & SLACK, 1969), which indicate that even under normal conditions flight durations tend to be short, though a proportion of flies captured on bait animals had reached the point of proline exhaustion; and with the observations of VALE

(1973) on the response of tsetse flies to moving baits, which indicate that individual members of the following male swarm only remain with the swarm for a few minutes.

A further indication of the close relation between proline availability and flight activity is provided by the observation of HARGROVE (1973) that the progressive decline in proline concentration during flight is associated with a corresponding fall in wing beat frequency, which drops from an average of 220 beats per second at initiation of flight to about 180 after 2–3 minutes. This suggests that the performance of the flight system may be limited by the rate at which energy can be supplied to it, as set by the availability of substrate. The affinity of the proline dehydrogenase system for its substrate is low (BURSELL & SLACK, 1970) and for maximal activity high concentrations would require to be maintained at the mitochondrial surface. As the haemolymph concentration falls the supply of proline to the sarcosomes appears to become inadequate to maintain full saturation of the enzyme system, and as a result there is a fall in the rate of oxidation and hence in the rate at which energy is supplied for the contractile system.

These metabolic limitations have important implications in relation to aerodynamic performance; the lift force which can be produced by the flight system has been shown to be proportional to the square of the wing beat frequency (HARGROVE, 1973) and a decline in frequency will therefore be associated with a marked decline in the capacity to carry load. The substantial lift force which would have to be developed by a newly-fed fly at take-off could probably only be sustained for a matter of seconds, and it is not surprising, therefore, that such flies have difficulty in gaining height, and "tend to fly horizontally to the nearest tree where they rest for more than half an hour" (GLASGOW, 1961). This period would allow time for primary excretion to be completed and for the load of the bloodmeal to be reduced by half (BURSELL, 1960). During later, and lighter, stages of the hunger cycle the energy required to keep the fly airborne would then be capable of being supplied on a continuing basis until the stage of proline exhaustion is reached.

3. Uses of the bloodmeal

The central role of proline in the metabolism of tsetse flies has been outlined in the previous section, and attention may now be turned to a consideration of the relationship between the metabolic system and the bloodmeal which constitutes its main input, and to the manner in which that raw material is used to sustain the various requirements of the system as a whole.

(a) For growth and reproduction

The adult life of the tsetse is characterised by an early growth phase, associated with the maturation of the flight system (BURSELL, 1961a; LANGLEY, 1970; BURSELL & KUWENGWA, 1972; HARGROVE, 1973). This involves an increase in the residual dry weight of the thorax, the process extending over the first two hunger cycles in males, and the first three in females, as illustrated in Fig. 4a. During this time substantial quantities of contractile, mitochondrial and cuticular proteins are laid down and the blood meal serves as the raw material for these growth processes. Until development has been completed the flight capabilities of the insects are relatively feeble, maximal wing beat frequencies for teneral flies being 190 compared with 230 for mature flies at initiation of flight. The correspondingly low lift capacity of teneral flies appears to be associated with a tendency to take small blood meals, as illustrated in Fig. 4b. For male flies the first two blood meals would provide a total of 8 mg dry weight of protein, of which 1.2 mg, or 15 %, would have to be set aside for the production of cuticular and muscle proteins; the balance would be available to meet the energy and other requirements of the organism.

In female tsetse flies demands on the blood meal for material, as distinct from energy, continues into maturity since provision has to be made for the intrauterine development of larvae. This development is sustained predominantly on the basis of the excretion of a uterine 'milk', rich in proteins, amino acids and fats (CMELIK et al., 1969). With the average intake of blood during an 11-day pregnancy (4 meals each of 50 mg weight) at about 40 mg dry weight and the average dry weight of a fully developed larva at about 7 mg (see HOFFMANN, 1954), approximately 18 % of the total intake of dry matter would have to be diverted to the purposes of reproduction, not counting the metabolic cost of transforming the raw material into larval substance.

It is interesting to note that the heavy requirement for raw material posed by the growth of the flight apparatus appears to be associated with a delay in the initiation of other activities which demand a heavy investment of material. The work of JACKSON (1953) has shown that under natural conditions the build-up of fat reserves in males of *G. morsitans* does not begin until late in the second week of adult life (see Fig. 4c), by which time the development of thoracic musculature would have been largely completed. In females, the well-known delay in the descent of the first egg to the uterus, which results in larviposition on about the 16th day at 25°, compared with subsequent 11-day interlarval periods, should perhaps be seen as a similar phenomenon. By the time demands arise for uterine secretion to nourish the first larva,

the thoracic musculature would have undergone the bulk of its development (see Fig. 4d).

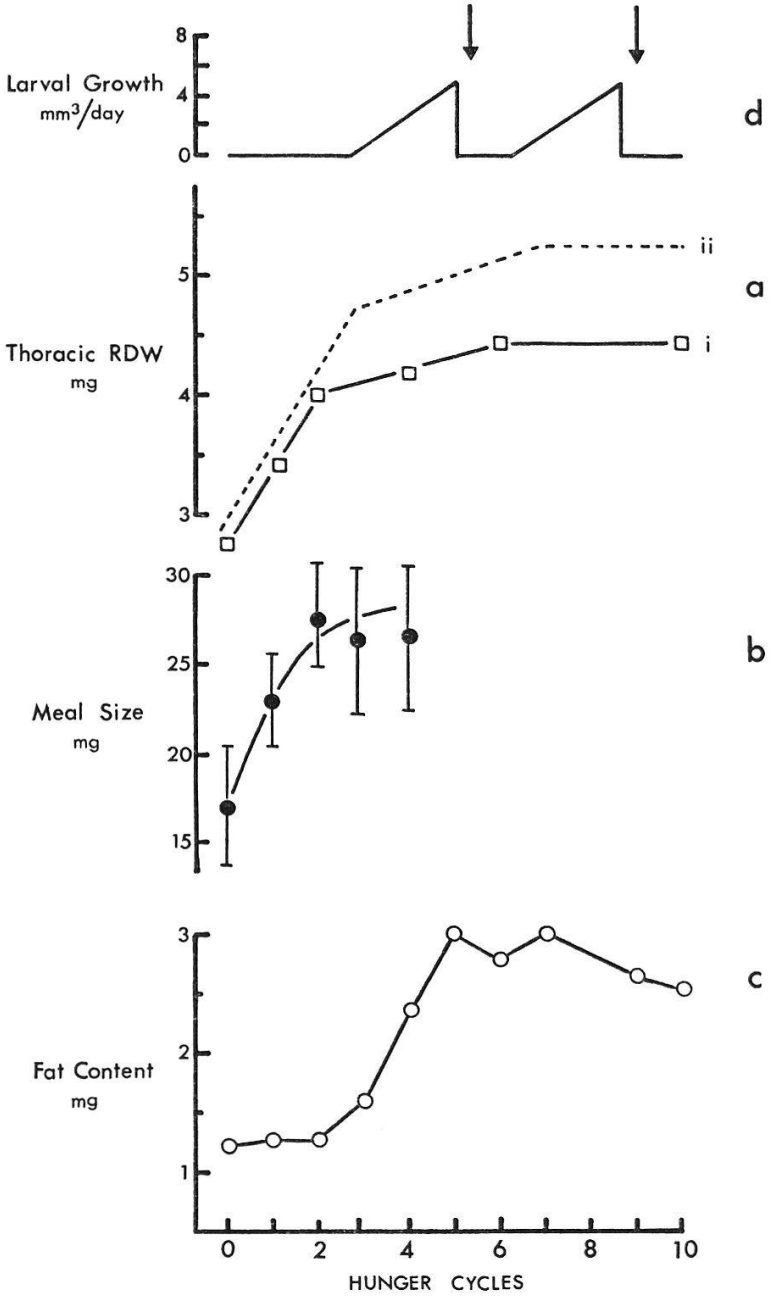

Fig. 4. The development of flight musculature in tsetse flies *(Glossina morsitans)* and associated phenomena. (a) The increase in thoracic residual dry weight in males (i) and females (ii). Data from BURSELL (1961a) and (1973). (b) Weight of human blood taken in successive blood meals by male flies. From unpublished data. (c) The fat content of males following release from pupae in the natural environment. Data from JACKSON (1953). (d) The timing of secretory activity in the female milk gland, as judged by the increase in volume of larvae during successive instars. Based on data in HOFFMANN (1954) and ROBERTS (1972). Time of larviposition indicated by arrows. A three-day hunger cycle has been assumed.

In view of these considerations and of the likelihood that starvation is an important cause of death in natural populations of tsetse (BURSELL, 1961b) it would seem that the early life of the tsetse may be a time of particular vulnerability. Because the flight system is poorly developed only small blood meals can be obtained from the first and second host encounters, and of these small blood meals a high proportion must be devoted to growth, leaving little to spare for the energy reserve. The time available to the fly for finding its hosts would be correspondingly limited, and the chances correspondingly smaller of replenishing food resources before they have become exhausted.

(b) For lipid synthesis

Once the requirements for growth and reproduction have been met, the balance of the blood meal may be used for the synthesis of food reserves, mainly lipid, and for provision of the energy required for maintenance of the system as a whole. The synthesis of lipids from blood meal amino acids has been investigated by MCCABE (1973) from whose work it appears that, with the exception of certain amino acids specifically involved with excretion (see section 3e below), the rest contribute to synthesis of the lipid reserve roughly in proportion to the number of carbon atoms that they contain, synthesis being routed mainly through acetyl CoA as the common precursor of fatty acids, and with C16 and C18 fatty acids predominating in the triglyceride end product (see also CMELIK et al., 1969). The synthetic machinery is only active during early stages of the hunger cycle, lipid synthesis ceasing after about 24 hours despite the presence of an abundant supply of raw material (see section 4 below); the control mechanism involved has not yet been identified.

It must be emphasized that the synthesis of fatty acids, and their esterification with glycerol to form the ultimate storage product, are processes which require a substantial input of energy and of reducing power in the form of NADPH. The metabolic cost of synthesising one molecule of tripalmitate, a common storage form for the tsetse, using glycerol and acetyl CoA as raw material, would be about 24 molecules of ATP (GREEN & ALLMAN, 1968), and to sustain it would therefore require the partial oxidation of 1.7 molecules of proline. This energy would be conserved in the product and would become available when the triglyceride is subsequently oxidised, but what is of importance in the light of results to be discussed in a later section is that the synthesis of triglyceride will of necessity be associated with a high rate of oxidative metabolism.

(c) For energy release

A constant feature of McCABE's (1973) work on the incorporation of radioactivity following administration of C^{14}-amino acids is that high levels of incorporation in proline are observed, whatever the amino acid. It is clear that there is a partial diversion of amino acids arising as products of digestion to provide substrate for the oxidative machinery of the resting fly, and that proline is of central importance during rest as well as during flight. The mechanism of incorporation into proline will differ from one amino acid to another, with many entering at points on the Krebs' cycle (e.g. glutamate, valine, aspartate – see Fig. 1). Others would be incorporated by way of acetyl-CoA (e.g. leucine and lysine), with lipogenic and oxidative pathways competing for this common intermediary during early phases of the hunger cycle.

(d) For proline synthesis

The ready incorporation into proline of carbon from amino acids other than alanine suggests that re-synthesis of proline may occur by pathways different from that discussed in section 2f, where alanine was the only amino acid precursor. Recruitment of a range of amino acids for proline synthesis would be metabolically convenient in that it would short-circuit the synthetic pathway involved in the production of lipids, and the energy input associated with that pathway. From the data provided by LANGLEY (1966) and McCABE (1973) it can be calculated that during the main digestive phase of the hunger cycle amino acids would be released at a rate of approximately 200 μg/h, sufficient to make a major contribution to the re-synthesis of proline following fight depletion, which has been shown to occur at the rate of about 100 μg/h (BURSELL, 1963); and since the release of digestive products appears to be potentiated by flight activity, it is even possible that these alternative pathways might supplement the reconstitution of proline from lipid reserves during flight to the point where a substantial augmentation of the proline reserve might result. During late stages of the hunger cycle, when the rates at which proline precursors are released from the residual blood meal becomes inadequate to sustain the requisite rate of synthesis, it is clear that lipids would have to play a major part in proline reconstitution; but at other times other substances may predominate as an input to the metabolic system.

(e) For nitrogenous excretion

The use of protein as a source of energy raises a difficulty in that the nitrogen that it contains must be removed before it can be fully

oxidised. It has been shown that the main vehicle of nitrogenous excretion is uric acid, as would be expected in a terrestrial insect, with arginine, histidine and haematin as additional excretory materials (BURSELL, 1965b). The two amino acids, and the prosthetic group of haemoglobin, have particularly high nitrogen contents, rivalling that of uric acid itself, and it would be metabolically uneconomical to deaminate them since the slight metabolic gain which could be derived from deaminated products would be offset by metabolic losses involved in the disposal of their nitrogen by synthesis of uric acid. It is not surprising, therefore, that these substances appear to be quantitatively eliminated in the excreta. The possibility that arginine may play an active part in flight metabolism is suggested by a transient increase in its concentration soon after initiation of flight (BURSELL, 1963), and by the fact that it becomes quite strongly labelled with C^{14} administered before flight as proline or glutamate (BURSELL, 1966), but the point of its participation remains to be discovered.

The results of MCCABE (1973) indicate that uric acid synthesis in tsetse flies proceeds by normal pathways, with CO_2, formate and glycine as the main carbon precursors. The amount of glycine, and of its main precursor, serine, that arises during digestion is inadequate to account for the amount of uric acid synthesised and it is likely that the supply of carbon is supplemented by conversion of alanine to glycine through serine.

It can be calculated that for every mg of dry weight ingested by the fly, almost a half must be excreted in order to dispose of the surplus nitrogen in the ways described (BURSELL, 1965b). Since 14 % of the uric acid molecule is derived from CO_2, the ultimate oxidation product, this may slightly overstate the position, but the material equivalent of the energy required to power uric acid synthesis must also be taken into account; one proline molecule would need to be completely oxidized to provide energy for the synthesis of 5 molecules of uric acid. It is clear that not much more than 50 % of the blood meal could become available as a source of energy, a fact that strikingly underlines the difficulties involved in the combination of a blood-sucking habit with a uricotelic excretory metabolism.

(f) As a source of carbohydrate

Mammalian blood contains about 0.1 % carbohydrate, and a blood meal of 30 mg would provide approximately 70 μg of glucose. The existence of glycogen deposits in various tissues of the tsetse fly (D'COSTA, RICE & LATIF, 1973) and the presence of enzymes involved in gluconeogenesis, albeit at low levels of activity (NORDEN & PATERSON, 1969, 1970), suggests that blood glucose may serve as a raw

material for the synthesis of glycogen, which reaches levels of about 110 μg/fly. It is of interest in this context that the secretory activity of Malpighian tubules of the tsetse cannot be sustained by proline, but requires glucose or pyruvate (Dr. M. J. Berridge, pers. comm.). It seems possible that the oxidative pathways that have been described in Section 2 may be peculiar to the flight muscle, and that some or all other tissue processes may meet their requirements for energy on the basis of substrates other than proline.

4. Metabolism through the hunger cycle

In this section an attempt will be made to fit the concepts developed above into the general context of the hunger cycle; for this purpose the changes that occur during the hunger cycle in various rate processes, and associated fluctuations in the concentration of various substrates, as illustrated in Fig. 5, will form a useful basis. It must be emphasized, however, that the data on which this illustration has been based were obtained with flies maintained under laboratory conditions, whose digestion is known to be much slower than that of field flies (LANGLEY, 1967), and that the time course of changes in natural populations of tsetse might be correspondingly faster.

The first day of the hunger cycle is seen to be characterised by intense metabolic activity, as reflected in the sharp rise in oxygen consumption which occurs as soon as a blood meal is taken (Fig. 5a); rates of digestion (Fig. 5b), and of excretion, increase rapidly and lipid synthesis rises to an early peak about 12 hours after the meal (Fig. 5c). It is clear that the high rate of oxygen consumption may be taken as a reflection of a high requirement for energy by the active processes involved, particularly in the synthesis of fats and of uric acid. The total quantity produced during the period in question is 1.2 and 1.5 mg respectively (BURSELL, 1963 and 1965b), and using the values given in sections 3b and 3e for the energy requirement involved, it can be calculated that the synthesis would be associated with the consumption of about 340 μl of oxygen. This represents nearly 80 % of the increment in respiration over basal levels, as demarcated by the hatched portion of Fig. 5a.

The low proline contents which characterise this "lipogenic" phase of the hunger cycle (see Fig. 5d) would be in accord with the view that proline constitutes the main substrate for oxidative metabolism; in spite of the rapid release of proline, and of proline precursors, from the digestive tract, there is no sign of a progressive build-up, indicating that the substrate is oxidised as fast as it is produced. The fact that alanine concentration shows an increase during this period (Fig. 5e) suggests

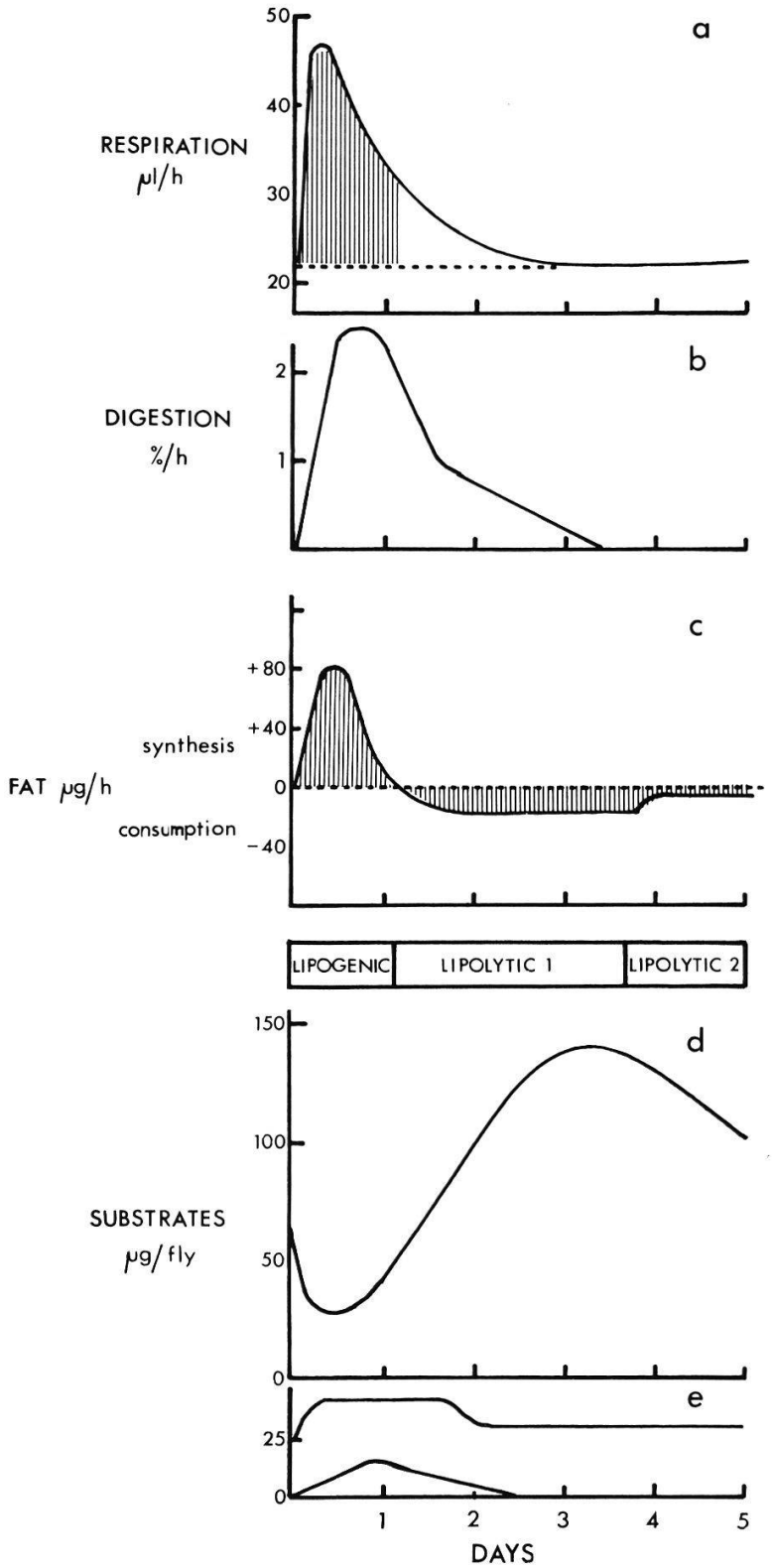

Fig. 5. Changes in rate processes and in substrate levels during the hunger cycle of *Glossina morsitans* males maintained in the laboratory. (a) The rate of oxygen consumption. Data from RAJAGOPAL & BURSELL (1966). (b) The rate of digestion, expressed as a percentage of the total blood meal digested per hour. Data from LANGLEY (1966). (c) The rate of change in the level of lipid reserves, positive values indicating synthesis, negative values utilisation. Data from BURSELL (1963). (d) Changes in the level of proline, and (e) in the levels of alanine (upper curve) and leucine (lower curve). Data from MCCABE (1973) and BURSELL (1963). For further explanation see text.

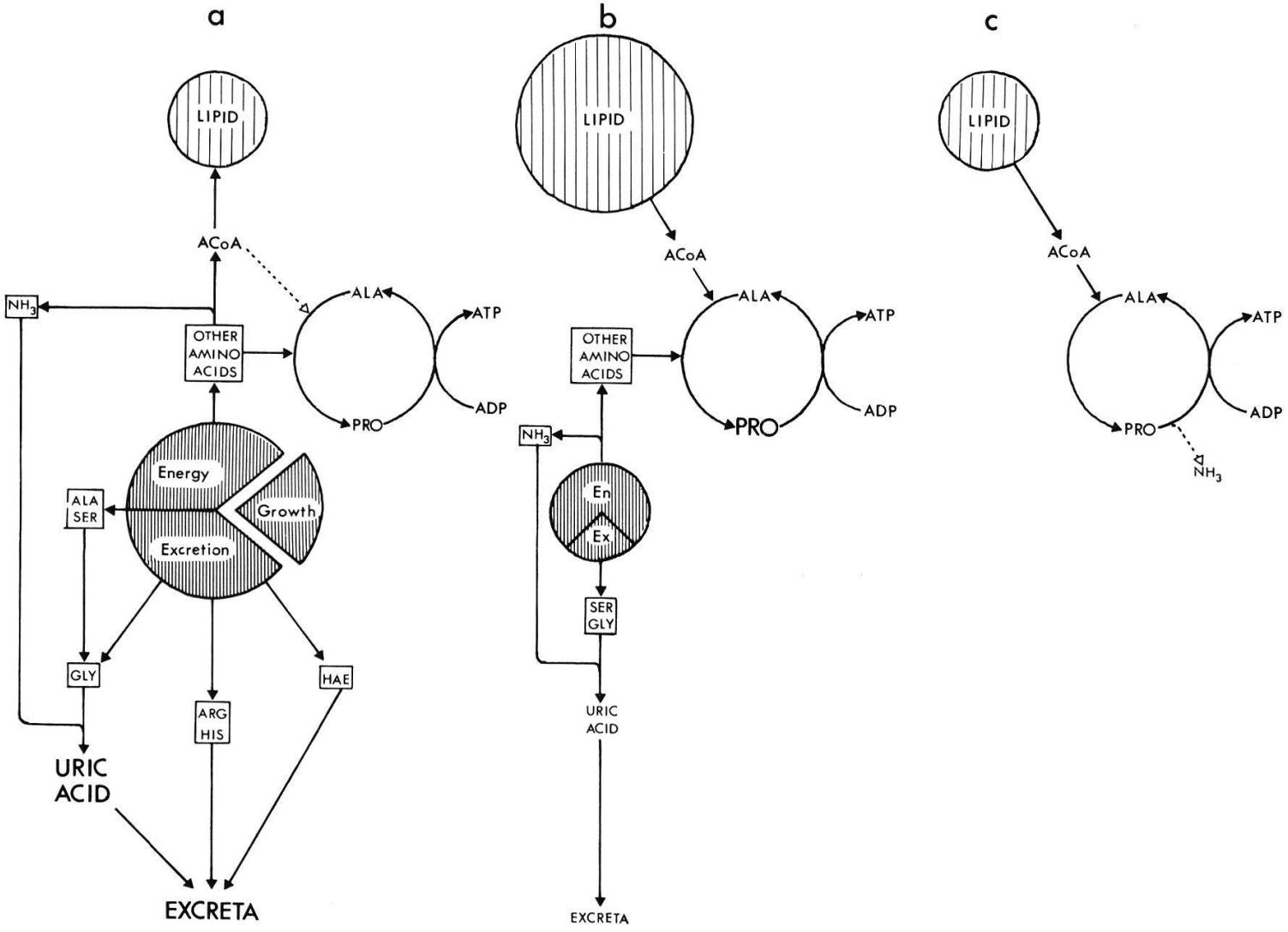

Fig. 6. A summary of metabolic patterns at different stages of the hunger cycle. (a) *The lipogenic phase:* the blood meal constitutes the main source of energy, after materials have been set aside for growth and development; of the remainder about half has to be devoted to the elimination of surplus nitrogen, a process which involves the 3-carbon amino acids (glycine, serine and alanine) together with arginine, histidine and haematin, uric acid being the principal excretory product. Other amino acids constitute an input to the proline pool, and raw material for the synthesis of lipids. (b) *The first lipolytic phase:* fat reserves have been replenished and serve as a source of raw material for energy production; the proline reserve is building up, and losses of nitrogen through the activity of glutamic dehydrogenase are made good from amino acids of the residual blood meal; demands on this source for the excretion of surplus nitrogen have been correspondingly reduced. (c) *The second lipolytic phase:* the residual blood meal has been exhausted, and lipids constitute the sole input to the proline pool; the proline reserve shows progressive depletion due to losses of nitrogen associated with flight. For further explanation see text.

that even in the resting fly transaminases are active and proline oxidation is partial rather than complete. The concentration of some of the other amino acids, such as leucine, increases as the rate of digestion

increases (Fig. 5e), suggesting that during periods of maximal digestion the rate at which they appear in the haemolymph is somewhat greater than the rate at which they can be converted to oxidisable products.

The processes which are supposed to predominate during lipogenesis have been schematised in Fig. 6a. The blood meal has been partitioned into three main portions, variable according to the stage of development of the insect which will determine what proportion of the total requires to be set aside for growth and development. Of the remainder, half is assigned to excretion through the main agency of the 3-carbon amino acids, with the bulk of arginine, histidine and haematin excreted directly and so immune from metabolic manipulation. Proline is figured as the starting point of the energy-yielding oxidative process and alanine, the product of its partial oxidation, is shown as precursor of the reconstitutive pathway. Other amino acids are shown as precursors of acetyl CoA, the raw material of lipid synthesis, and as contributors to the proline pool either directly or through acetyl CoA as an intermediary.

Following the initial lipogenic phase there is a sharp fall in the rate of respiration, which drops to basal levels as processes of digestion and excretion are completed. As the partial oxidation of proline slows the alanine concentration drops (see Fig. 5e) and proline levels show a progressive increase to reach a peak at about 150 μg/fly on the fourth day of the hunger cycle. During this time the lipid reserve begins to be used, indicating that a substantial proportion of the total energy requirement is being supplied from this source, and the phase may therefore be described as "lipolytic". Predominant processes have been schematised in Fig. 6b, which indicates that most of the energy reserve is at this stage stored in the fat body, and the residual blood meal makes up a relatively small proportion; pathways associated with the disposal of surplus nitrogen have been correspondingly attenuated. The energy-yielding oxidation of proline is shown to be sustained by input from amino acids of the residual blood meal, and from lipid reserves.

The last metabolic phase of the hunger cycle is characterised by a reduction in the rate at which fat stores are depleted (see Fig. 5c), associated with the fall to basal levels of the resting rate of respiration; and with a progressive decline in the concentration of proline. At this time there is nothing left of the residual blood meal and therefore no source of supply for amino nitrogen from which losses of nitrogen associated with the activity of glutamic dehydrogenase could be made good. Under these circumstances the insect may be considered as subject to progressive nitrogen depletion, which would have serious consequences against the background of a metabolism based on the oxidation and reconstitution of proline. For every molecule of nitrogen lost, one less molecule of alanine is produced, and one less molecule of

proline can be synthesised. In this way successive bursts of flight activity would cause a partially irreversible depletion of the proline reserve and a point would eventually be reached where flight activity would be totally inhibited by low proline levels, while resting metabolism could still be maintained on the basis of the nitrogen-conserving transaminase pathways.

The situation during this last phase of the hunger cycle is schematised in Fig. 6c, where metabolism is seen to be sustained solely by an input from depleted lipid reserves, and with the system as a whole subject to progressive attenuation associated with losses of nitrogen which would occur during flight.

In view of the fact that flight capacity appears to be limited by the availability of proline, as discussed in section 2g, it is interesting to note that the changing proline levels illustrated in Fig. 5d appear to be associated with corresponding changes in the general level of activity. The newly-fed fly is known to be extremely inactive, in accord with the very low concentration of proline which characterises this phase of the hunger cycle; it is likely that available concentration of substrate would be inadequate to furnish energy at a rate commensurate with the high lift force which would need to be generated for flight of an insect carrying a heavy blood meal. BRADY (1972) has shown that under laboratory conditions there is a progressive increase in the proportion of time spent active during the next three days, which correlates well with the progressive increase in the proline reserve. Unfortunately his experiments with fed flies do not extend beyond the 5th day and direct experimental evidence for a progressive decrease in flight activity associated with the later fall in proline concentration in mature flies is lacking. But the wholly flightless condition of flies in later stages of starvation is a phenomenon which will be familiar to most tsetse workers, and this could perhaps be regarded as the ultimate expression of such a trend.

5. Conclusions

For purposes of the present review care has been taken to confine attention to those results which can reasonably be interpreted on the basis of the proposed model, and this will have given the impression that there are few loose ends. That is far from being the case. Many of the observations made by different workers cannot convincingly be accommodated within the framework of the concepts developed in this review; one thinks particularly of the secondary increase in glutamate during flight (HARGROVE, 1973) for which no satisfactory explanation has been found; of the fact that aspartate appears to be an active participant in metabolism, to judge by the fact that when radioactive

amino acids are administered a substantial proportion of radioactivity is invariably associated with this substance, and its specific activity is often high, yet it features nowhere among the postulated metabolic pathways; the metabolic role of arginine still remains uncertain and as yet no work has been done on the fat body, to discover whether it has the enzymatic capabilities to play the part which has been postulated for it on the basis of circumstantial evidence. It is clear that until these matters have been resolved the suggestions that have been put forward concerning the pattern of tsetse metabolism must remain provisional.

For all that, there can be little doubt that the metabolism of the tsetse shows many peculiar features which can reasonably be seen as metabolic adaptations to a blood-sucking mode of life. In so far as their basis has been identified they appear to involve changes which are essentially quantitative; enzymes which are common to many insects but relatively feebly developed, such as proline dehydrogenase, being extremely active in the tsetse, while the reverse is true of others, as with the enzymes of glycolysis; and the cumulative effect of such substantial quantitative differences is to produce a pattern of metabolism which one might consider as qualitatively different from that recognised as characteristic of other Diptera, and of insects generally. The pattern could perhaps be best characterised by saying that it is dominated by proline, the partial oxidation of which provides for most of the energy needs of the insect. The apparent inability of the flight system to oxidize any other substrate at rates commensurate with flight requirements places a limitation on its performance, in the sense that when the limited proline reserve is exhausted, flight must cease. Seen in the context of species biology, however, this limitation would seem to be more apparent than real. The life of the tsetse fly is characterised by limited movement based on intermittent, rather than sustained, activity of the flight system (BURSELL, 1970) and for this the metabolic system would seem to be perfectly adapted.

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