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Nutrition of *Glossina morsitans*: Metabolism of U-^{14}C Threonine during Pregnancy

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Abstract

Following injection of U-^{14}C threonine into the haemolymph of adult female *Glossina morsitans* during late pregnancy, radioactivity was detected in the post-parturient female and in its offspring, in threonine, lipids, and a range of non-essential amino acids. The level of radioactivity recovered from the larva was higher than that remaining in the injected adult, and the radioactivity recovered was considerably higher in the amino acid than in the lipid fraction.

Administration of labelled threonine into maternal haemolymph on each of the first 8 days of the 9–10 day long pregnancy cycle was followed 24 h later by measurement of radioactivity in the developing oöcyte and in the intra-uterine progeny. The patterns of nutrient uptake are discussed in relation to vitellogenesis in the oöcyte and to growth of the larva. Analysis of the expired carbon dioxide and excreta was carried out 24 h after maternal injection of labelled threonine on the first or eighth day of pregnancy. Carbon dioxide and excreta from females in early pregnancy showed significantly higher radioactivity than those from females in late pregnancy. In both cases, radioactivity in the amino acid fraction from the excreta was extremely small and about 95% of the total activity was in uric acid. These results are discussed in terms of the utilization of threonine in relation to the metabolic demands for various nutriments by the pregnant female.

Introduction

In haematophagous *Glossina* the mode of reproduction is adenotrophic viviparity, and hence the normal growth of the intra-uterine progeny is a function of optimum feeding and efficient utilization of bloodmeal nutrients by its female parent. The adult female *G. morsitans* takes approximately 200 mg of food during a pregnancy cycle (Bursell et al., 1974). The dry weight of this quantity of blood from a goat, the host used for the routine feeding of *G. morsitans* in this laboratory, is 37.6 mg of which at least 84% is protein (Moloo, 1976a). Since proteins constitute the main nutrient in the diet, metabolism of those amino acids which are essential for the maintenance of nitrogen equilibrium and the promotion of growth in this insect (Moloo et al., 1974), are being investigated. The aim is to obtain information on the nutrition of the adult female and to apply the results to the development of an efficient artificial feeding system. Threonine is an essential constituent of the diet of *G. morsitans* (Moloo et al., 1974), and the present study examines metabolism of this nutriment in the adult female during the intra-uterine development of progeny.

Materials and Methods

Flies

G. morsitans were obtained from the self-supporting goat-fed colony maintained in this laboratory (NASH et al., 1971). The experiments were undertaken with female flies in their 9–10 day long second pregnancy cycle. Females were grouped after formation of the tanned polypneustic lobes in their intra-uterine first larva, and those which larviposited within the following 24 h were designated day 1 of the second pregnancy cycle. All experimental flies were fed on goats and kept at 25 °C.

Synthesis of lipids and amino acids

Adult females were individually injected through the thoracic cuticle with 2 μl of U-^{14}C threonine (sp. act., 232 mCi/m-mol; radioactive concentration, 0.05 μCi/μl; Radiochemical Centre, Amersham) on the seventh and again on the eighth day of pregnancy. After larviposition, 10 post-parturient females and their recently pupariated larval offspring were washed with 0.4% lithium carbonate solution and then with several changes of distilled water to remove the adhering adult excreta. After surface drying, the two groups were chopped up separately in chloroform-methanol mixture (3:1) and lipids extracted for 24 h. The determination of ^{14}C-activity in chloroform-soluble lipid fractions was carried out as previously described (MOLOO, 1976b). After lipid extraction, the tissue debris from each sample was hydrolysed in 6N HCl in a sealed tube under nitrogen gas at 120 °C for 24 h. The hydrolysates were filtered (Whatman No. 1), dried *in vacuo,* and each redissolved in 1 ml of 50% methanol. After passing through a membrane filter (Millipore, 0.45 μm), 10, 20 and 50 μl samples were chromatographed using phenol and borate buffer (pH 8.6) mixture (4:1 v/v) in the first dimension (descending), and a n-butanol-acetic acid-water mixture (4:1:5 v/v) in the second (ascending). Amino acids were located with 0.2% ninhydrin in acetone, individual fractions cut out, each eluted in 1 ml of 50% methanol, and ^{14}C-activity measured as described previously (MOLOO et al., 1974). In this and in subsequent experiments ^{14}C-activity was measured with a Nuclear Chicago (Model 6850) scintillation counter, using NE 250 (Nuclear Enterprises Ltd., Edinburgh) scintillant, appropriate quench corrections being made with an internal standard.

Uptake of nutriments by the oöcyte and by in utero progeny

Seven flies on each of the first 8 days of pregnancy were individually injected with 2 μl of labelled threonine. Twenty-four hours later, the most mature oöcyte in the ovary (i.e. the right outer, see SAUNDERS 1960) and the uterine content of each fly were carefully removed, washed in several changes of physiological saline, each solubilized *in toto* in 0.6 N NCS tissue solubilizer (Amersham/Searle Corp.), and ^{14}C-activity determined as previously described (MOLOO, 1976b). To compare the rate of uptake of threonine and its synthetic products by the three intra-uterine larval instars, pregnant females of appropriate physiological age were injected as before and, after varying periods from 20 to 120 min, total ^{14}C-activity in each larva was determined as described earlier.

Excretory products of threonine metabolism

Ten females each, on the first or the eighth day of their second pregnancy cycle, were individually injected with 2 μl of labelled threonine and kept in environmental containers. Twenty-four hours later, radioactivity in the expired carbon dioxide from each fly was measured as previously described (MOLOO, 1976b). Excreta from 5 flies were individually eluted with 5 ml of 0.4% lithium carbonate solution, and 1 ml aliquot from each sample was brought to about pH 7 with acetic acid and total ^{14}C-activity measured. Excreta from the remaining 5 flies were similarly individually eluted but with 5 ml of distilled water, amino acids separated using a column of Zerolit 225, and radioactivity determined. Details of the general procedure have been described elsewhere (MOLOO, 1976b).

Results

Table 1 shows the distribution of ^{14}C-activity in various amino acid fractions and lipids from each post-parturient female and its larval offspring following injection of labelled threonine into females on the

Table 1. Distribution of ^{14}C-activity in various amino acid fractions and lipids from the post-parturient female and its larval offspring after injection of U-^{14}C threonine into pregnant female *G. morsitans*

Fraction	Female		Offspring	
	counts/min	%	counts/min	%
Alanine	610	0.8	2,040	2.8
Arginine	–	–	–	–
Aspartic acid	400	0.5	2,210	3.0
Cystine/Cysteine	120	0.2	210	0.3
Glutamic acid	640	0.9	3,000	4.1
Glycine	610	0.8	2,470	3.4
Histidine	–	–	–	–
Leucine/Isoleucine	–	–	–	–
Lysine	–	–	–	–
Methionine	–	–	–	–
Phenylalanine	–	–	–	–
Proline	850	1.2	3,200	4.4
Serine	510	0.7	2,320	3.2
Threonine	3,800	5.2	34,480	47.5
Tryosine	–	–	–	–
Valine	–	–	–	–
Amino acid total	7,540	10.4	49,930	68.8
Lipids	3,320	4.6	11,800	16.3
Total	10,860	15.0	61,730	85.0

seventh and eighth day of pregnancy. Radioactivity is expressed as counts/min/fraction and as percentages of total recovery from the two life stages. The total ^{14}C-activity recovered in amino acids from the post-parturient female and from its offspring were 71% and 83% of the activity recorded in their respective hydrolysates. The total amount of the labelled material in aqueous solution injected into each fly was 4 μl which gave 3.6×10^5 counts/min, and contained 0.11 μg of threonine. The total activity recovered from the post-parturient female and its offspring was 0.85×10^5, that is 23.6% of the administered activity. The major proportion of the remaining activity represents metabolic losses due to expired carbon dioxide and excretion. The amount of threonine injected into each female was extremely small in relation to the total amount of free amino acids present in the haemolymph (LANGLEY & PIMLEY, 1974), and hence the addition of such small amount is unlikely to have affected the normal course of physiological events in the fly.

In addition to the injected radioactive threonine, the amino acids alanine, aspartic acid, cystine, glutamic acid, glycine, proline and serine showed ^{14}C labelling. Arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tyrosine and valine showed no threonine-derived ^{14}C incorporation. Tryptophan is destroyed by acid hydrolysis and hence was not detected. The radioactive amino acids recovered from the post-parturient female and from its offspring were identical, but the total activity in these nutrients in the latter was markedly higher. A similar trend was observed in the case of the radioactive lipids. Of the non-essential amino acids the highest activity was recovered in the proline fraction followed by glutamic acid. The radioactivity in threonine was 52.7%, in the non-essential amino acids 26.4%, and in lipids 20.8% of the total recovered activity.

Table 2 shows total ^{14}C-activity in the developing oöcyte and in the intra-uterine progeny 24 h after injection of pregnant females with labelled threonine, on each of the first 8 days of pregnancy. The amount of ^{14}C-activity in the developing oöcyte increased to a maximum on day 5–6 and then rapidly declined: the amounts detected were extremely small and varied between 0.09% and 0.49% of the injected activity.

During the first 4 days of pregnancy the intra-uterine egg showed no ^{14}C-activity (Table 2). Figure 1 shows the relationship between ^{14}C-activity in each of the three larval instars and the period following labelled threonine injection of their female parents; each point represents the mean of 5 observations. Following eclosion, the first instar showed ^{14}C-activity, but the rate of uptake of the radioactive nutriments was extremely low ($b = 0.009$; $t = 12.9$, $P < 0.001$). There was then a very rapid increase in the radioactive labelling of the intra-uterine

Table 2. Radioactivity in the oöcyte and in the intra-uterine progeny 24 hr after U-^{14}C threonine injections into adult female *G. morsitans* on different days of the 9–10 day long second pregnancy cycle

Day of pregnancy females injected	Oöcyte		Intra-uterine progeny	
	Counts/min $\bar{X} \pm$ SE	Percent of injected activity	Counts/min $\bar{X} \pm$ SE	Percent of injected activity
1	165 ± 6	0.09	–	–
2	228 ± 11	0.13	–	–
3	380 ± 15	0.21	–	–
4	478 ± 21	0.27	1,957 ± 99	1.1
5	887 ± 38	0.49	10,090 ± 544	5.6
6	294 ± 14	0.16	36,531 ± 1,904	20.3
7	257 ± 12	0.14	77,999 ± 3,370	43.3
8	163 ± 4	0.09	74,308 ± 2,836	41.2

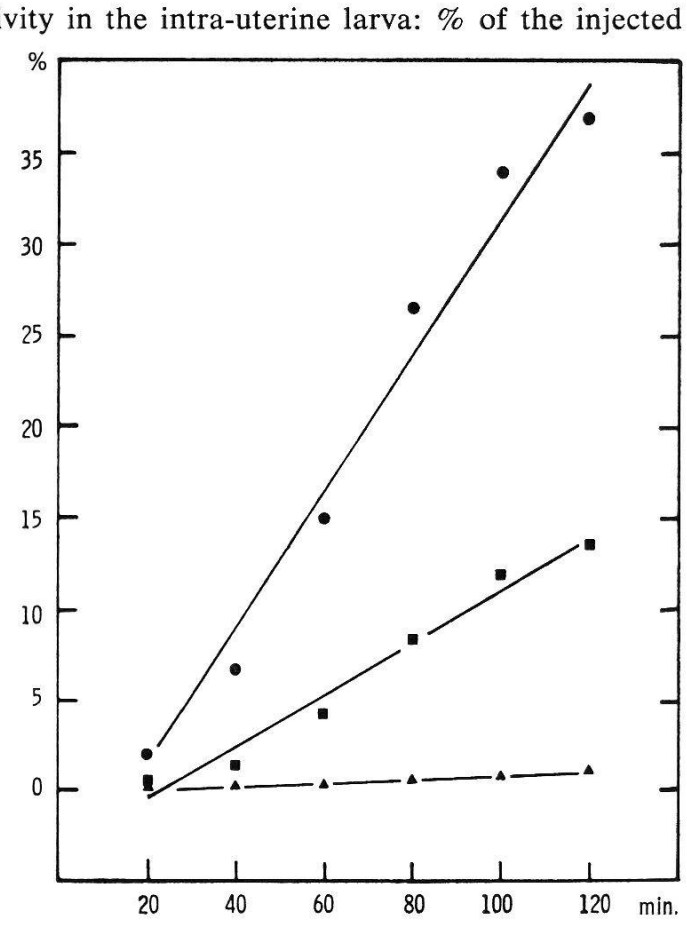

Fig. 1. Relationship between total ^{14}C-activity expressed as percentage of injected activity in the three larval instars and time following labelled threonine injection into female parent. In each case the relationship is linear: ▲–▲, first instar, y = 0.009x –0.2; ■–■, second instar, y = 0.15x –3.39; ●–●, third instar, y = 0.384x –6.51.

progeny as development continued, reaching a maximum when the third instar larva appeared. Ecdysis to the second instar was associated with a sixteenfold increase in the rate of uptake of threonine and its synthetic products ($b = 0.146$; $t = 14.5$, $P < 0.001$). The change to the third instar was followed by the most rapid uptake of the labelled nutriments; the slope ($b = 0.384$; $t = 13.3$, $P < 0.001$) of the linear regression indicates a two- to threefold increase in the rate of nutriment uptake by this instar over that by the previous instar. The ^{14}C-activity in each of the three instars 24 h following the injection of the female parent was only marginally higher than that recovered 2 h after such injection. Hence, the activity shown in Table 2 represents radioactive nutriment uptake by the three instars for only a short period of the 24 h following injection of the female parent.

After the labelled threonine injection of the females on day 1 or 8 of pregnancy, the expired carbon dioxide collected for 24 h gave $29{,}982 \pm 1{,}253$ (mean ± S.E.) and $10{,}480 \pm 539$ counts/min/fly, respectively. The difference is highly significant ($t = 14.3$, $P < 0.001$). The above radioactivities are 16.6% and 5.8% of the injected counts. Labelling of the excreta from day 1 pregnant females was also higher than that from females in late pregnancy. Radioactivity in the excreta from the former flies was $77{,}470 \pm 1{,}793$ counts/min/fly, that is 43% of the injected counts. Of this mean activity, 95.8% was in uric acid and 4.2% in amino acids. In the case of females in late pregnancy, the mean activity in excreta was $53{,}440 \pm 2{,}403$ counts/min/fly, that is 29.6% of the injected counts, and 96% was recovered in uric acid and 4% in amino acids. Thus the activity in amino acids excreted by pregnant females of two different physiological age groups were proportionally similar, but the total recovery of labelled uric acid from females in early pregnancy was significantly greater than in late pregnancy ($t = 9.5$, $P < 0.001$). The activity in the excreta from females in late pregnancy was 69% of that from females in early pregnancy.

Discussion

The present study has shown that following injection of labelled threonine into the haemolymph of adult female *G. morsitans* during late pregnancy, in addition to the administered threonine, lipids and the non-essential amino acids alanine, aspartic acid, cystine, glutamic acid, glycine, proline and serine from both post-parturient female and its larval offspring showed ^{14}C labelling. Of the total radioactivity recovered in the above nutriments from these two life stages, 52.7% was in threonine, 26.4% in the non-essential amino acids, and 20.8% in lipids. Total radioactivity in the above amino acids was markedly

higher in the post-parturient third instar larva than in its injected female parent: of the total activity in these nutriments 86.9% was in the larva as against 13.1% in the female parent. Synthesis of proteins and their transfer to the growing intra-uterine larva has been demonstrated in *G. austeni* (TOBE & DAVEY, 1974); this is probably the case in *G. morsitans* also, although in the present study ^{14}C-activity was determined in various amino acids from female flies and their larval offspring after hydrolysis and no attempt was made to determine such activity in their original proteins. Radioactivity detected in the lipid fraction from both the stages of this insect is suggestive of lipid synthesis from threonine, and its utilization for larval nutrition. However, of the total radioactivity in the post-parturient third instar larva 80.9% was in amino acids and only 19.1% in lipids. This suggests that during the most rapid growth of the intra-uterine larva which occurs after ecdysis to the third instar during late pregnancy (DENLINGER & MA, 1974; LANGLEY & PIMLEY, 1975), amino acids transported to the haemolymph are utilized for synthesis of proteins to a greater extent than of lipids for larval nutrition. During early pregnancy, however, threonine transported to the maternal haemolymph is utilized for various metabolic activity but the surplus threonine is largely converted to lipids and, in addition to their utilization to meet the parental metabolic demands, these storage lipids and to a smaller extent amino acids are transported to the intra-uterine larva sometime during the second half of the pregnancy period (MOLOO, 1976c). Hence, the maternal metabolic system is such that during early pregnancy there is a greater synthesis of larval lipids than of larval proteins from the bloodmeal threonine transported to the maternal haemolymph, but latterly there occurs a change to the synthesis of predominantly larval proteins. A similar pattern of larval nutrition was observed in the case of another amino acid leucine (MOLOO, 1976c).

The recovery of threonine-derived labelled lipids and amino acids from the post-parturient female is of interest, and suggests that apart from providing for metabolic requirements of the overall system and for larval growth a significant proportion of the bloodmeal nutriments remain and, following larviposition, serves to sustain the female fly until it finds its next host. It has been shown that this insect is resistent to abortion (SAUNDERS, 1972), but since the pregnancy period is temperature-dependent such resistence is only limited. Hence, if for some reason food intake is suboptimum the fly either produces smaller offspring or aborts its uterine content (MELLANBY, 1937). The intra-uterine larva is dependent upon its female parent for all its needs, so that survival of the female fly is of greater significance in terms of species survival. Hence under conditions of nutritional deficit the fly would utilize its reserves for its own metabolic activity and the nutri-

tion of its intra-uterine larva would be adversely affected. The confinement of the greatest growth of the larva to late pregnancy is possibly of particular significance in this context. During early pregnancy the female fly builds up nutriment reserves from its bloodmeals (MOLOO, 1976c). If the total meal intake during this period is small the amount of storage nutriments would be correspondingly low. Should such nutriment deficit prevail for a certain critical period the fly would abort its uterine content and continue host-seeking activity. Although the incidence of abortion in nature is unknown, it is conceivable that in areas with a general scarcity of natural hosts food shortage might thus play a role in regulating population density.

The uptake of threonine and its synthetic products by the oöcyte on different days of development concur with vitellogenesis and growth of the oöcyte (MOLOO, 1971; DENLINGER & MA, 1974). Following ovulation, the next most mature oöcyte has already undergone cytological differentiation of its trophocytes and formation of its follicle layer, and for the next four days the oöcyte slowly increases in size with progressive yolk deposition. There then follows a period of the most rapid vitellogenesis and growth of the oöcyte lasting for about two days, after which the rate of yolk deposition drops and continues to decline. Utilization of threonine and its synthetic products for oöcyte development is very low on the eighth day when the trophocytes undergo degenerative changes, and ceases completely on the ninth day when chorionation is completed. Hence, growth of the oöcyte is in synchrony with *in utero* development of the progeny, so that following larviposition one egg is already fully developed and soon descends into the uterus to undergo further growth and development.

The uptake of threonine and its synthetic products by the intra-uterine progeny is also in close agreement with its growth (DENLINGER & MA, 1974; LANGLEY & PIMLEY, 1975) and with the uptake of glucose and glucose-derived nutriments by the three larval instars (MOLOO, 1976b). However, the intake of threonine and its synthetic products is greater than that of glucose and its products: the rate of uptake of the former by the first, second and third instars were respectively 1.8, 7.7 and 6.4 times greater than that of the latter nutriments (see MOLOO, 1976b). Also, after labelled glucose injection of the pregnant female, radioactivity in the larva was largely in lipid and non-essential amino acid fractions, the activity in the glucose fraction being exceedingly small. It has been demonstrated that proteins and lipids form the main constituents of the fully-fed third instar larva (CMELIK et al., 1969; MOLOO, 1976a), and since carbohydrate-based metabolism is poorly developed in this insect (BURSELL et al., 1974), a much greater utilization of threonine for larval growth reflects the nature of metabolic system operative in the pregnant female.

The present results also suggest that threonine transported to the maternal haemolymph during early pregnancy is utilized to a significantly greater extent for the provision of metabolic energy than that transported at the time of the most rapid growth of the intra-uterine third instar larva. Movement of the experimental flies was restricted by the small size of the experimental chamber, and hence radioactivity recorded in the excretory products was in the main from inactive flies. It has been demonstrated that during early pregnancy surplus threonine is largely converted to storage lipids (MOLOO, 1976c), a biosynthetic process which requires a substantial input of energy (BURSELL et al., 1974). During late pregnancy, however, the emphasis shifts to protein synthesis, and so at the time of the most rapid larval growth the net direction of threonine metabolism is towards synthesis of specific uterine milk proteins rather than oxidation. Nevertheless, oxidative catabolism of threonine and/or of its synthetic products continue, though the amount thus utilized is now smaller. During late pregnancy in addition to other physiological activities the female fly nourishes the intra-uterine third instar larva at an appreciably higher rate resulting in the most rapid larval growth. It is thus most likely that the overall energy demand of the female at this time is higher than during early pregnancy, and that in the main substrates other than threonine provide oxidative energy.

After injection of labelled threonine into the haemolymph of adult females on the first or the eighth day of pregnancy, radioactivity in the amino acid fraction from the excreta was extremely small and about 95% of the total activity was in uric acid. This agrees with the observation of BURSELL (1965) but conflicts with the suggestion of LANGLEY & PIMLEY (1974) that amino acids are transferred to the larva only at the time of its rapid growth but at other times of pregnancy these nutriments are excreted unchanged. Indeed, if this were the case the excretion of large amounts of bloodmeal-derived amino acids would represent a great wastage of otherwise useful nutriments and would not reconcile with the principle of maximum economy inherent in living organisms. The adult female has a significant capacity to store surplus nutriments for larval growth (MOLOO, 1976c), and the present study serves to illustrate that in adult female *G. morsitans* the metabolic system is such that there is a great conservation of nutriments to provide for its own requirements as well as for its intra-uterine progeny.

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References

BURSELL, E. (1965). Nitrogenous waste products of the tsetse fly *Glossina morsitans*. – J. Insect Physiol. *11*, 993–1001.

BURSELL, E., BILLING, K. C., HARGROVE, J. W., MCCABE, C. T. & SLACK, E. (1974). Metabolism of the bloodmeal in tsetse fly. – Acta trop. *31*, 297–320.

CMELIK, S. H. W., BURSELL, E. & SLACK, E. (1969). Composition of the gut contents of third instar larvae (*Glossina morsitans* Westwood). – Comp. Biochem. Physiol. *29*, 447–453.

DENLINGER, D. L. & MA, W. (1974). Dynamics of the pregnancy cycle in the tsetse *Glossina morsitans*. – J. Insect Physiol. *20*, 1015–1026.

LANGLEY, P. A. & PIMLEY, R. W. (1974). Utilization of U-^{14}C amino acids and U-^{14}C protein by adult *Glossina morsitans* during *in utero* development of larva. – J. Insect Physiol. *20*, 2157–2170.

LANGLEY, P. L. & PIMLEY, R. W. (1975). Quantitative aspects of reproduction and larval nutrition in *Glossina morsitans morsitans* Westw. (Diptera, Glossinidae) fed *in vitro*. – Bull. ent. Res. *65*, 129–142.

MELLANBY, H. (1937). Experimental work on reproduction in the tsetse fly, *G. palpalis*. – Parasitology *29*, 131–141.

MOLOO, S. K. (1971). Oöcyte differentiation and vitellogenesis in *Glossina morsitans* Westw. – Acta trop. *28*, 334–339.

MOLOO, S. K. (1976a). Aspects of the nutrition of adult female *Glossina morsitans* during pregnancy. – J. Insect Physiol. *22*, 563–567.

MOLOO, S. K. (1976b). Nutrition of *Glossina morsitans:* metabolism of U-^{14}C glucose during pregnancy. – J. Insect Physiol. *22*, 195–200.

MOLOO, S. K. (1976c). Storage of nutriments by adult female *Glossina morsitans* and their transfer to the intra-uterine larva. (In press).

MOLOO, S. K., LANGLEY, P. L. & BALOGUN, R. A. (1974). Amino acid synthesis from glucose-U-^{14}C in *Glossina morsitans*. – J. Insect Physiol. *20*, 1807–1813.

NASH, T. A. M., JORDAN, A. M. & TREWERN, M. A. (1971). Mass rearing of tsetse flies (*Glossina* spp.): recent advances. – In Sterility Principle for Insect Control or Eradication, pp. 99–110. Vienna: I.A.E.A.

SAUNDERS, D. S. (1960). The ovulation cycle in *Glossina morsitans* Westwood (Diptera; Muscidae) and a possible method of age determination for female tsetse flies by examination of their ovaries. – Trans. R. ent. Soc. Lond. (A) *42*, 221–238.

SAUNDERS, D. S. (1972). The effect of starvation on the length of the interlarval period in the tsetse fly *Glossina morsitans orientalis* Vanderplank. – J. Ent. (A) *46*, 197–202.

TOBE, S. S. & DAVEY, K. G. (1974). Autoradiographic study of protein synthesis in abdominal tissues of *Glossina austeni*. – Tissue & Cell *6*, 255–268.