STUDIES ON THE ROLE OF SERUM PROTEINS IN THE OVARIAN FUNCTION OF THE TSETSE FLY, *GLOSSINA PALPALIS PALPALIS*

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W. Takken

STUDIES ON THE ROLE OF SERUM PROTEINS IN THE OVARIAN FUNCTION OF THE TSETSE FLY, *GLOSSINA PALPALIS PALPALIS*

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
hoogleraar in de organische scheikunde,
in het openbaar te verdedigen
op vrijdag 19 december 1980
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen

ISN: 122979-03

Aan mijn ouders

STELLINGEN

NN08201, 831

1. Serum albumine van runderen is een essentieel voedingsbestanddeel voor het vrouwtje van de tsetse vlieg, *Glossina p. palpalis* (Rob.-Desv.).

Dit proefschrift.

2. De afgifte van het diurese hormoon aan de haemolymph in *Glossina* spp. wordt positief beïnvloed door de aanwezigheid van glucose in het dieet.

Dit proefschrift.

3. De observatie van Langley *et al.* dat *Glossina m. morsitans* Westwood, indien gevoed op een dieet van erythrocyten en een zoutoplossing, larven produceert, dient verder onderzocht te worden.

Langley, P.A., Pimley, R.W., Mews, A.R. en Flood, M.E. (1978). J. Insect Physiol. 24, 233-238.

4. Er bestaat een duidelijke behoefte aan een geautomatiseerd systeem van organisatie voor biologische parameters teneinde zo effectief mogelijk insekten te kunnen kweken op grote schaal.

5. De wetenschappelijke belangstelling voor slechts enkele soorten van het genus *Glossina* kan leiden tot een onderschatting van het sociaal-economische belang van de overige soorten.

Tobe, S.S. and Langley, P.A. (1978). Ann. Rev. Entomol. 23, 283-307.

6. De zorgvuldigheid waarmee de bestrijding van de iepespintkever, *Scolytus* spp., dient plaats te vinden teneinde tot enig resultaat te leiden, is omgekeerd evenredig met de zorgvuldigheid die betracht wordt bij de houtsoortenkeuze.

7. Natuurbeheer is een min of meer gecontroleerde vorm van verwaarlozing.

Beheerstoestand van het bos op de Veluwe. Rapport Dorschkamp no. 145.

8. De wereld is uit haar evenwicht, gezien het feit dat die groep van de wereldbevolking welke dringend behoefte heeft aan bevolkingsgroei regulerende middelen, veel minder toegang heeft tot die middelen dan de groep welke reeds beschikt over een gereguleerde bevolkingsgroei.

9. Het geeft te denken dat de ontwikkeling van methoden ter eliminatie van chemische produkten ver achter blijft bij de snelheid waarmee deze produkten gesynthetiseerd worden.

10. Luchtvaartmaatschappijen zouden geen betaling voor overgewicht moeten verlangen van passagiers die zelf lichtgewicht zijn.

W. Takken

Studies on the role of serum proteins in the ovarian function of the tsetse fly, *Glossina palpalis palpalis*

Wageningen, 19 december 1980

preface

At the completion of this dissertation, I would like to thank dr. M. Fried, director, Joint FAO/IAEA Division of Radiation and Isotope Applications of Atomic Energy for Food and Agricultural Development, Vienna, Austria, for the opportunity given to work on this thesis during my assignment at the Joint Division and for the many encouraging discussions held about the subject. I also would like to thank drs. P. Vail and G.L. LaBrecque, the former as initiator and both as great supporters of this study. Special thanks to dr. H. Wetzel, whose initiative and great support helped to solve many bottlenecks. Drs. C.O. Calkins, T.G.T. Jaenson, L. Gringorten, A. van der Vloedt and M. Weiss are thanked for the many stimulating discussions, their advice and the sometimes spiritual help. Jennifer Elliot, Kaarina Kiviranta Kada and Martina Herz, who all assisted me at one time for shorter or longer periods, thank you for the endurance shown, especially with the lipid extractions. I thank in particular the fly breeding staff, Mrs. H. Fröhlich and M. Niedrist and Messrs Fröhlich, Hafenscher, Ivantschitz and Acs. Without you, the foundation of the laboratory, all our tsetse studies would not have been possible. Prof.dr. J. de Wilde, whose encouragement at the initiation and during this study was felt as a support from abroad, is thanked, in particular for the many helpful discussions and suggestions. Finally, I would like to thank my wife, Françoise Kaminker, whose support and comfort given at the completion of this thesis were often strengthening and very helpful.

contents

general introduction

The genus *Glossina* belongs to the group of obligatory haematophagous insects. Several of its species are of great socio-economic importance as vectors of African trypanosomes, the causative agents of sleeping sickness in humans and nagana in domestic animals. To lower the risk of trypanosome infections, various methods of tsetse fly control have been widely applied (Ford, 1970; Mac Lennan, 1967; Jordan, 1978). In recent years a new method of fly control has been developed, in which sterile male flies are released in a natural fly population to compete with wild males for mating and insemination of females (Knipling, 1973; Cuisance *et al.*, 1978; Dame and Williamson, 1979). This sterile insect release method (SIRM) requires the availability of relatively large quantities of sterile insects reared at lowest cost possible since the economic feasibility of SIRM will be greatly determined by the cost of each released and sterile fly.

The successful development of a membrane-feeding technique for tsetse flies (Bauer and Wetzel, 1976; Mews *et al.*, 1976, 1977) can be considered as a major breakthrough in the mass-rearing studies of this insect. Recently, a technique to rear *Glossina palpalis palpalis* (Rob.-Desv.) on blood which was reconstituted after being lyophilized and stored until used, was reported (Wetzel, in press). However, the obligatory blood feeding behaviour of tsetse would continue to make tsetse mass-rearers dependent on suitable host animals to provide blood (Mews *et al.*, 1977; Wetzel and Luger, 1978; Bauer and Aigner, 1979). An artificial diet, such as is known for many other non-blood sucking insect species (Vanderzant, 1974) would be desirable. Such a diet could also be used by workers studying life cycles and infection mechanisms in *Trypanosoma* spp.

To date, artificial diets for obligatory haematophagous insects have not been reported though physiological studies on feeding behaviour (e.g. food-acceptance, -intake and -diges-

tion) have been published and reviewed (Lall, 1969; Akov, 1972; Freyvogel (ed.), 1975; Langley, 1976; Friend and Smith, 1977). The metabolic pathway of various digested particles in haematophagous insects has been investigated and for *Glossina* particularly the complete pathway of amino acids, glucose and some fatty acids is well understood (Bursell *et al.*, 1974; Tobe and Davey, 1974; Langley and Pimley, 1974; Moloo *et al.*, 1974; Moloo, 1976a, 1976b, 1977a, 1977b; Tobe, 1978; Langley and Bursell, 1980). Though some blood feeding insects might be autogenous and can be reared on simple diets, not containing amino acids, most blood feeding insects appear to require some sort of a protein source in order to complete oogenesis and follicle development. Various mosquito species can be kept alive on sucrose and water, but they need blood proteins (or amino acids) for egg production (Greenberg, 1951; Lea *et al.*, 1956).

In order to develop an artificial diet for a blood feeding insect, it seems useful to understand the feeding behaviour and to reveal the essential factors (those elements that cannot be produced by the insect's own metabolic pathways) in the food medium. The present study was undertaken to examine various essential nutritive elements in the meal of female *G. p. palpalis*. Article 1 deals with meal size and primary excretion as a function of diet composition. In article 2 the effect of serum proteins on fecundity and offspring size is described. Article 3 discusses the effect of serum albumin on the reproductive system, in particular the follicle development. The relationship between follicle development and lipid storage as a function of diet composition is discussed in article 4.

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Influence of diet composition on meal size and rate of excretion of the tsetse fly *Glossina palpalis palpalis*

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Abstract

The suitability of various diets for the tsetse fly *Glossina palpalis palpalis* has been investigated in terms of food uptake and primary excretion. Serum substitutes were prepared reflecting the composition of bovine serum and offered to teneral female flies, on a few occasions in combination with erythrocytes. Routinely, all diets contained 10^{-3} M ATP. There was no difference in meal size when flies were fed on serum or serum-substitutes, alone, or in combination with erythrocytes. The rate of primary excretion was reduced in diets, where serum was substituted by an isotonic saline containing bovine albumin or bovine albumin + gamma-globulin. Glucose, added to a diet containing albumin, induced a normal excretion rate. In all cases a combination of erythrocytes and sera (-substitutes) caused a reduced primary excretion as compared to the sera (-substitutes) alone. The rate of excretion was highly correlated with the meal size, regardless of the diet composition.

Introduction

The rearing of tsetse flies (*Glossina* spp.) in the absence of living host animals (the so-called *in vitro* technique) has been successful in recent years (Bauer & Wetzel, 1976; Mews *et al.*, 1977). The reported systems all require regular supply of fresh vertebrate blood which has been prevented from clotting. In the *in vivo* system, tsetse flies can be fed on various different types of host animal, e.g. goat, rabbit of guinea pig (Itard & Jordan, 1977). Using the *in vitro* system, the flies do not thrive on all types of vertebrate blood. *Glossina palpalis palpalis* (Rob.-Desv.) has been successfully reared on fresh defibrinated bovine blood but cannot be maintained on equine blood (Wetzel & Luger, 1978). Conversely, *G. morsitans morsitans* Westwood does not thrive on fresh defibrinated bovine blood, whereas it performs well on porcine and equine blood (Mews *et al.*, 1976, 1977; Wetzel & Luger, 1978). Satisfactory explanations for these nutritional differences have not yet been found.

For the establishment of large tsetse fly colonies in Africa for use in sterile-insect-release-method (SIRM) programmes, it is expected that, for economic reasons, the *in vitro* system will be applied. However, the collection of fresh blood from the preferred host animal might be of disadvantage under tropical conditions especially because of the risk of contamination with trypanosomes. The availability of an instant diet (e.g. freeze dried blood or a blood substitute) therefore seems necessary for the large scale application of SIRM.

To date, nor artificial diet is known to be succesful for an obligatory haematophagous insect like the tsetse fly. As a first step in the preparation of such a diet, it would seem useful to take note of the composition of vertebrate blood and then test the flies on artificial diets resembling blood in composition. As the tsetse fly is a relatively slow breeder (a female fly will produce only one offspring every 9 to 10 days), it is reasonable as a first step to determine whether dietary sub-

stances are acceptable in terms of the physiology of food-uptake and of the highly efficient excretion process of excess dietary water (Lester & Lloyd, 1928). In this study, the effects of several serum substitutes on the feeding response, food-uptake (Friend & Smith, 1977) and primary excretion (Moloo & Kutuza, 1970) of *G. p. palpalis* are reported.

Material and Methods

Flies used in this study were all teneral (= previously unfed) females and originated from puparia of a membrane-fed colony of *G. p. palpalis* (Wetzel & Luger, 1978). The flies were 24 to 48 hrs old at the time of feeding. With few exceptions, each experimental solution was presented to 30 flies taken at random.

The food uptake and subsequent urine production were measured by the weight changes in the flies after feeding. Prior to feeding, each fly was weighed in a tared open-ended plastic vial (6 cm x 4 cm diam.) covered with terylene netting. The vial was then placed on the feeding unit (Bauer & Wetzel, 1976). Feeding was considered to be finished after withdrawal of the proboscis from the membrane. Flies which excreted during feeding were discarded. Each fly that took a meal was weighed immediately thereafter and again 1 and 3 hrs later. Prior to weighing, it was transferred to a clean vial in order to exclude the weight of the excreta. Since the test-flies did not excrete during feeding, the difference in weights of the flies prior to and immediately after feeding is a reliable measure of the amount of diet ingested. All handling took place in a controlled environment (25°C; 70% R.H.).

Experimental solutions (serum substitutes) were prepared, based on the composition of bovine serum (Table I). Bovine blood was chosen as the reference food medium because the *G. p. palpalis* colony at Seibersdorf is routinely fed on this blood source.

All test solutions were made up from a solution with the following composition: 100 mM NaCl; 5 mM KH_2PO_4; 40 mM $NaHCO_3$. The pH of this solution varied between 7.4 and 8.0. It was isotonic with bovine serum and will be referred to as 'saline'. Enrichment of the saline was obtained by adding bovine serum albu-

min (BSA, Cohn fraction V), BSA + gamma-globulin (SERVA, Heidelberg, FRG) or BSA + glucose. A detailed composition of the experimental solutions is shown in Table I. As a feeding stimulus adenosine-5'-triphosphate (ATP) was added routinely to all test-solutions in a concentration of 10^{-3} M (Galun & Margalit, 1969; Langley, 1972). Serum and red blood cells (RBC) were obtained from fresh, defibrinated bovine blood; on the day of collection, serum and cells were separated by centrifugation in a clinical centrifuge at 2500 rpm for 20 minutes. The serum was then drawn off, leaving the cell mass. Serum, RBC and the experimental solutions were never older than 7 days when they were offered to the flies. All solutions were stored in the refrigerator at 4°C prior to use. The flies were fed on experimental solutions alone or on a combination of exp. sol. and RBC (50/50 by volume). Flies fed on serum or serum + RBC (50/50 by volume) served as controls in all experiments.

The pH and osmolarity of each solution were checked at regular intervals; pH-readings were made using a Beckman digital pH-meter (model 3550) with combination electrode no. 39504; osmolarity was measured using a Knauer electronic halfmicro osmometer Type M (Knauer Wissenschaftliche Geräte KG, PB 1322, 6470 Oberursel, FRG).

Unless otherwise stated, results were analysed by Student's t-test procedure for testing differences between means.

Results

pH and osmolarity

Data on one-day old solutions of serum substitutes alone or in combination with RBC are presented in Table II. Without RBC, the osmolarity of the solutions tended to increase with increasing BSA concentration, exceeding 300 mOsm/kg at 9 g % BSA. In all other cases, the osmolarity was not much different from that of serum. Gamma-globulin (1 g %) and glucose (0.07 g %) influenced the osmolarity only slightly. The pH-value of saline was reduced by about 0.3 by the addition of BSA; but the pH was largely independent of the albumin concentration in the diet. The addition of RBC to any solution appeared to have a stabi-

Table I: The composition of bovine serum and serum-substitutes, tested for feeding reactions of *G. p. palpalis*

Chemical component	Bovine[1] serum	Serum-substitutes			
		Saline	Saline + BSA	Saline + BSA + -globuline	Saline + BSA + glucose
H_2O	91				
Proteins	6.4-7.1		4.5-9.0[3]	5.5-7.0[3]	4.5
Albumin (g/100 ml)	3.0-5.0		4.5-9.0[3]	4.5-6.0[3]	4.5
Globulins (g/100 ml)					
α-	0.5-1.0				
β-	0.6-1.2				
γ-	0.7-1.5			1.0	
Non-protein N (mg/100 ml)	31				
Urea (mg/100 ml)	22				
Amino acids (mg/100 ml)	50				
Carbohydrates (mg/100 ml)	40-70				70
Lipids (mg/100 ml)	348				
Organic acids (mg/100 ml)	12				
Vitamins & hormones	2)				
Inorganic - Cations					
Na^+ mM	141	140	140	140	140
K^+ mM	4.4	5	5	5	5
Ca^{2+} mM	2.5				
Mg^{2+} mM	1.2				
- Anions					
Cl^- mM	104	100	100	100	100
PO_4^{3-} mM	1.9	5	5	5	5
HCO_3^- mM	20	40	40	40	40
Trace elements	2)				

1) after Kolb, 1962; 2) present in very low concentrations; 3) varied according to test-medium.

Table II: Osmolarity and pH of serum-substitutes, alone and combined with RBC.[1)]

Medium	without RBC		with RBC (50/50)	
	Osmolarity mOsmol/kg	pH	Osmolarity mOsmol/kg	pH
RBC (washed)			292	7.53
Serum (= control)	287	7.86	285	7.71
Saline	274	8.05	272	7.75
Saline (4.5 g BSA/100 ml)	284	7.78	285	7.71
Saline (6.0 g BSA/100 ml)	283	7.72	287	7.74
Saline (9.0 g BSA/100 ml)	314	7.73	295	7.69
Saline (4.5 g BSA + 1.0 g γ-globulin/100 ml)	292	7.78	292	7.71
Saline (6.0 g BSA + 1.0 g γ-globulin/100 ml)	290	7.78	293	7.72
Saline (4.5 g BSA + 0.07 g glucose/ 100 ml)	290	7.80	289	7.71

[1)] one-day old solutions

lizing effect on pH and osmolarity. The osmolarity and pH of all RBC supplemented media were not much different from those of reconstituted blood (= RBC + serum). It was observed that during storage the pH-values of all solutions (including serum) increased slowly (e.g. serum: 7.86 to 7.95), but whenever RBC were present, this pH increase was less. This effect can be explained by the bicarbonate fraction in the fluids and the strong buffering capacity of haemoglobin and phosphates in the red cells.

Food-uptake and urine production

Table III shows the results of the food-uptake and urine production at one and three hours after feeding. For reasons of comparison, the weights of the teneral flies have also been included. Although the control flies tended to be heavier than the

Table III. Food-uptake and cumulative primary excretion of 24-58 hrs old, teneral *G. p. palpalis* females, which were fed on different diets. In all cases means values and standard errors are given.

Diet	n-[1]	Feeding response (%)	Teneral fly weight (mg)		Meal size (mg)		Cumulative loss after 1 hr (%)		Cumulative loss after 3 hrs (%)		Corr.[2] control
Serum (c_1)	20	67	19.5	0.6	33.9	1.6	62.6	1.1	67.1	1.4	
Serum (c_2)	17	57	19.7	0.5	27.8	2.2	63.7	1.3	69.8	1.3	
Saline	23	77	18.1	0.5	32.3	1.3	48.6**	1.9	70.1	2.8	c_1
Saline (4.5% BSA)	22	73	18.6	0.7	30.1	1.7	44.6**	1.7	59.7**	2.3	c_1
Saline (6.0% BSA)	25	83	18.1	0.4	31.8	1.6	50.3**	1.8	61.9**	1.2	c_1
Saline (9.0% BSA)	17	57	18.1	0.6	30.5	2.2	41.8**	1.3	51.2**	1.7	c_1
Saline (4.5% BSA + 1.0% gamma-globulin)	24	80	18.3	0.6	22.4*	2.0	28.6**	3.2	50.5**	4.7	c_2
Saline (6.0% BSA + 1.0% gamma-globulin)	20	70	18.7	0.7	31.3	1.8	47.3**	1.9	60.9**	1.9	c_1
Saline (4.5% BSA + 0.07% glucose)	21	70	18.3	0.6	29.4	1.7	63.9	2.6	68.0	1.2	c_2
RBC + serum (c_3)	17	85	18.6	0.7	29.2	1.4	42.8	1.9	45.0	2.2	
RBC + saline	19	95	18.2	0.6	29.6	2.1	40.4	2.4	45.2	2.9	c_3
RBC + saline (4.5% BSA)	13	65	19.4	0.5	28.8	1.8	44.0	2.7	50.0	3.0	c_3

1) Number of flies that took a meal
2) Refers to serum diets
* Mean significantly different from corresponding control (t-test, $P < 0.05$)
** Mean significantly different from corresponding control (t-test, $P < 0.01$)

experimental flies, the differences were not significant (t-test, $P > 0.05$). Similarly, the average fly weights of all the experimental groups were not significantly different from each other ($P > 0.05$). The feeding responses (percentage of flies that took a meal) varied between 57 and 95 per cent. Except for one case (saline + 4.5% BSA + 1% gamma-globulin), average meal sizes for the different diets were not significantly different from each other ($P > 0.05$). The meal size of flies fed on the diet with 4.5% BSA and 1% gamma-globulin was also significantly different from the corresponding control ($P < 0.05$). Flies which had been fed on a mixture of RBC and serum (-substitutes) ingested as much as their corresponding controls (Table III, last section). Again, there were no differences in meal size between control and the experimental diets ($P > 0.05$).

The bulk of the urine of flies fed on serum only, was produced by approximately one hour after feeding (Table III). This was also the case in a diet containing both glucose and BSA. In all other cases, completion of urine production was delayed. After one hour, urine production, following a diet of saline or saline containing BSA or BSA + gamma-globulin, was 20 to 54% lower than of a serum diet. However, after three hours the differences had become less, but were still significant ($P < 0.01$) for all diets containing BSA or BSA + gamma-globulin. Flies fed on saline or saline containing BSA and glucose, had lost similar quantities of urine to the corresponding control groups after 3 hrs.

The addition of RBC to serum, saline or saline containing 4.5% albumin resulted in a reduced primary excretion as compared with meals where no RBC were present (Table III). The reduction was significant ($P < 0.01$) after one hour with the serum and saline diets, but only after 3 hrs with the saline + BSA diet.

The cumulative urine production 1 and 3 hours after feeding was clearly correlated with the meal size in all cases. The co-efficients of correlation were significantly different from zero ($P < 0.001$) and linear regression lines could be calculated (Fig. 1). One hour after feeding the co-efficients of regression of but one diet (saline + BSA + glucose) were significantly smaller than those of the control diet (t-test, $P < 0.01$), but

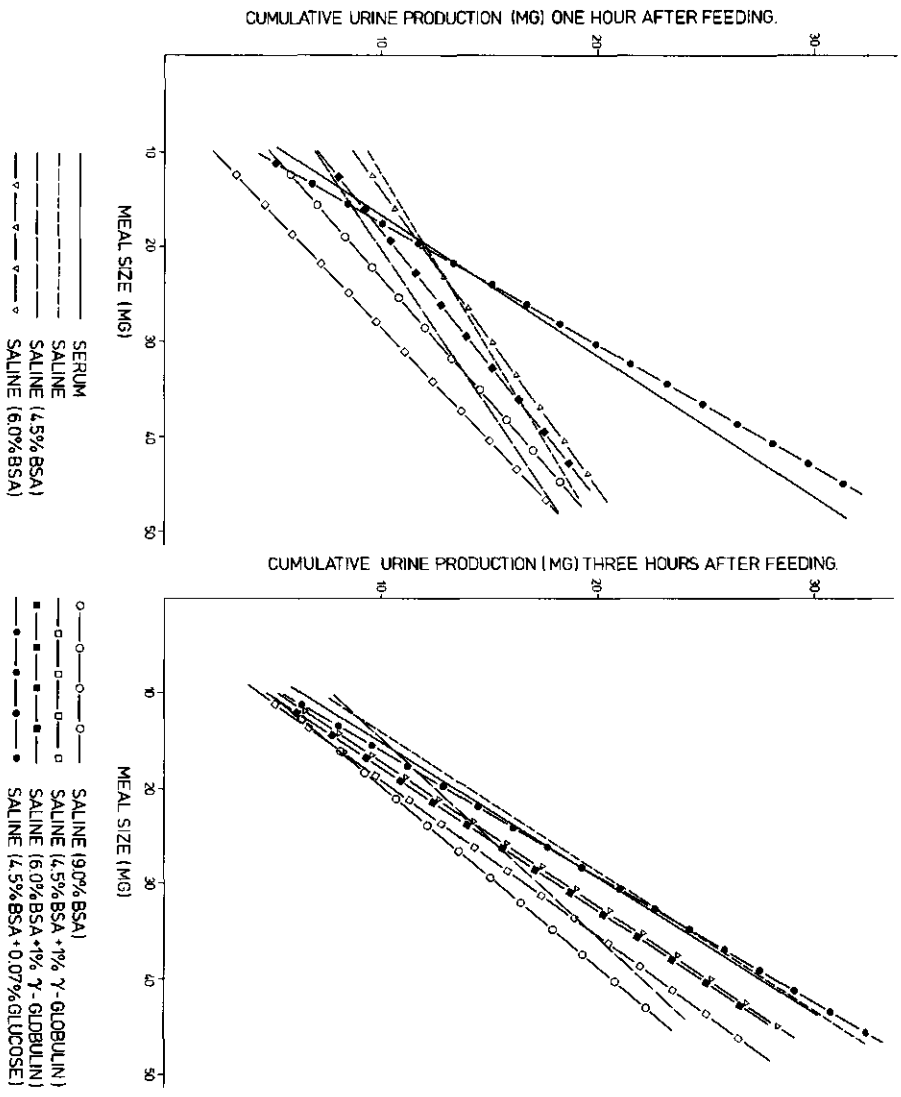

Figure 1. Calculated linear regression lines of urine production on meal size: a) one hour and b) three hours after feeding on serum or serum-substitutes.

they became very similar after three hours, except for the diets with 4.5% and 9% albumin. In these cases the differences in regression co-efficient from the control diets were still significant after 3 hrs ($P < 0.01$).

The regression lines of urine production on meal size with the RBC supplemented diets (Table III, lower part) have been omitted from Fig. 1. Linear regressions were significant ($P < 0.001$). The co-efficient of regression after a meal of RBC + saline was very similar to that of the control diet of RBC + serum; however, the addition of 4.5% albumin caused a significantly lower rate of excretion ($P < 0.01$) than that of the control diet 1 and 3 hrs after feeding.

Survival

In all experiments, none of the flies died or showed signs of paralysis during the observation period of 5 hrs.

Discussion

Tsetse flies will readily ingest solutions other than vertebrate blood provided a phagostimulant (preferably ATP) is present in the optimal concentration range (Galun & Margalit, 1969). Optimal ingestion rates in *Glossina* are only achieved with solutions of which the pH-values are close to that of verbrate blood (Langley, 1976). With one exception, the present investigations have demonstrated that on diets which approximate the composition of bovine serum, the meal size of female *G. p. palpalis* fed on defined diets was not different from that of flies fed on serum or whole blood. The significantly smaller food intake of a diet of 4.5% BSA + 1% gamma-globulin was unexpected and at present I have no explanation for it, because this result was not obtained when the BSA concentration was increased. The fact that none of the diets elicited a feeding response from all the flies, does not necessarily mean that the diets were *per se* unfavourable. It might have been due to the fact that the flies were contained singly in small vials. On a membrane-feeding system, *G. p. palpalis* will feed better when grouped together in a larger feeding cage where, it appears,

they stimulate each other to feed (Takken, unpublished). Those flies which fed all appeared to engorge to repletion.

From the results it may be concluded that *G. p. palpalis* does not require the presence of red blood cells in its diet for maximum ingestion. Thus, when the pH of a diet equals that of live-host blood and ATP is present in the optimal concentration, the flies engorge equally, regardless of the diet composition. Langley & Pimley (1973) found that the osmotic pressure of the feeding solution was of little importance in eliciting an ingestion response in *G. morsitans*, as long as ATP was present. However, water passage across the midgut-epithelium was influenced by the ionic concentration and composition of the diet. Tsetse flies frequently ingest meals which exceed as much as twice their body weight (Lester & Lloyd, 1928; Gee, 1975a). Fifty to 80% of the meal is temporarily stored in the crop but 5 to 20 min. after feeding, the crop contents have been emptied into the midgut (Moloo & Kutuza, 1970). This is possible because *Glossina* concentrates its meal by rapid diuresis. In this process, excess water passes from the anterior midgut into the haemolymph and is subsequently removed via the Malpighian tubules (Gee, 1975a). During diuresis, the composition of the haemolymph remains constant and the volume is not altered (Gee, 1975b; Tobe & Davey, 1972). Any differences in the ionic composition of a diet from vertebrate serum affect the rate of diuresis and the composition of the haemolymph, leading to paralysis and death (Gee, 1977). Therefore, the ionic composition of the test diets were kept close to that of serum.

The results on primary excretion of *G. p. palpalis* fed on serum or on whole blood agree well with the findings of Langley & Pimley (1973), who fed *G. morsitans* on heparinized goat blood and plasma. In both cases, the urine production was less after feeding on whole blood, presumably as a result of the lower water content of the meal (91% in serum and 66% in whole blood). However, in the present investigation, the presence of bovine serum albumin (or BSA + gamma-globulin) in the diet resulted in a significant reduction in primary excretion even 3 hrs after feeding, which did not appear in the presence of glucose. Gee (1977) suggested that *Glossina* possesses receptors which monitor

not only the size but also the nature of the meal and thus regulate the release of diuretic hormone which controls the amount of water removed by the Malpighian tubules. The results suggest that one of these postulated receptors might well be positively responsive to glucose. In addition, the presence of proteins (saline vs. saline + BSA) reduced diuresis. As none of the flies, fed on a diet containing serum proteins without glucose, showed signs of paralysis or death, it can be assumed that the proteins had reduced the passage of water across the anterior midgut but did not seriously interfere with the normal excretory physiology of the insects.

In combination with erythrocytes, solutions of serum substitutes elicited a diuresis similar to that in flies fed on whole blood. Such diets contained proportionally less water than sera substitutes alone and consequently there was less excess water to be eliminated. Langley & Pimley (1973), feeding *G. morsitans*, concluded that erythrocytes did not affect the rate of water excretion. In terms of total amount of water ingested, excretion rates were similar for diets with or without RBC. This agrees well with the present results with *G. p. palpalis* fed on whole blood or a mixture of erythrocytes and saline. However, the addition of albumin to such a mixture reduced the rate of diuresis ($P < 0.01$) but not the total amount of urine produced. It appeared that the presence of albumin in the diet inhibited the elimination of dietary water, reducing the efficiency of the excretory process. Recent work (Takken, unpublished) has shown that the addition of glucose prevents this inhibitory effect of albumin as in solutions without red cells.

G. p. palpalis, fed through artificial membranes on different solutions, had a urine production which was highly correlated with the meal size. This was also found for *G. morsitans* (Langley & Pimley, 1973; Gee, 1977). This correlation was not changed in the presence of erythrocytes and is additional proof that in *Glossina* primary excretion is determined by the water content rather than by the volume of the meal.

It may be concluded that teneral *G. p. palpalis* can be induced to feed on solutions of serum substitutes which reflect the composition of bovine serum. Such a diet will elicit a nor-

mal gorging response. If such a diet contains erythrocytes, a diuresis which is similar to that observed with whole blood will follow. There is evidence that serum albumin (or even serum proteins) inhibit diuresis but this does not occur if the diet also contains glucose. In a future paper, the suitability of these diets as alternative food-sources for tsetse flies, will be described.

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Influence of serum albumin on fecundity and weight of the progeny of the tsetse fly, *Glossina palpalis palpalis*

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Abstract

Females of a membrane-fed colony of *G. p. palpalis* (food source: fresh defibrinated bovine blood) were fed on derivatives of bovine blood, in which the red cell fraction remained unchanged while the serum fraction was replaced by an artificial solution. This solution consisted of a simple saline, isotonic to serum and enriched by bovine serum albumin with increasing concentrations. Physical parameters such as pH and osmolarity of all media were similar to controls. The absence of serum albumin in the foodmedium resulted in sterility of the females and caused high mortality. The presence of increased levels of serum albumin in the medium increased the fecundity until it was similar to that of flies fed on the standard diet of fresh defibrinated bovine blood. The offspring size was positively correlated with the concentration of the serum albumin in the mother's diet. However, a high concentration of albumin (> 6%) resulted in increased adult mortality. Thus, the presence of optimum amounts of free serum albumin in bovine blood appears necessary for the development and production of viable larvae of the tsetse fly *G. p. palpalis*.

Introduction

The successful development of the so-called '*in vitro*' feeding technique for tsetse flies (*Glossina* spp.) has been reported recently (Mews *et al.*, 1976, 1977; Wetzel & Luger, 1978). However, in the system one still depends on a regular supply of vertebrate blood, which has been prevented from clotting. To date no artificial diet is known for an obligatory haematophagous insect like the tsetse fly. Using the *in vitro* technique, one can study the nutritional requirements of *Glossina* in detail, which eventually may lead to a well-defined artificial diet.

As a first step in the preparation of such a diet, it would seem useful to investigate the composition of vertebrate blood and then test artificial diets resembling blood in composition on the flies. In a previous paper (Takken, 1980) I have reported on some effects of various serum substitutes in the diet of *Glossina palpalis palpalis* (Rob.-Desv.) on ingestion and excretion. It was shown that the flies readily accepted diets of which pH, osmotic pressure and ionic composition were similar to that of fresh defibrinated blood. Provided erythrocytes were present in the diet, the flies did not have a reduced diuresis though erythrocytes were not required for optimal ingestion rates. In the present study the effects of serum substitutes on the survival and fecundity of female *G. p. palpalis* and the size of their offspring are examined. To investigate the particular role of serum proteins in the diet of *Glossina*, the diets under study all contain a constant fraction of erythrocytes as they provide the bulk of protein in vertebrate blood.

Material and Methods

Newly emerged females of *G. p. palpalis* were obtained from a membrane-fed colony, routinely fed on defibrinated bovine blood since March 1976. For each experiment, the females were

divided into groups of approximately 60 flies, four cages with 15 flies in each. Each cage served as a replicate within the group. An additional group of 60 females of the same emergence date as those in the experiment was treated similarly and served as a control to indicate any changes in the development of the flies with time. The females were mated on day 3 of their adult stage with 7-day old males, and were separated 2-3 days thereafter. Mortality and fecundity were recorded daily except Sundays. Puparia were weighed individually when they were 0-24 hrs old, except on Mondays, when those produced since Saturday were also included. The maintenance and feeding technique have been described by Wetzel & Luger (1978).

The experimental flies were fed on derivatives of bovine blood in which the red-cell fraction remained unchanged while the serum was substituted by artificial solutions. The basic solution consisted of a sterile, isotonic saline which contained 140 mM Na^+, 5 mM K^+, 100 mM Cl^-, 5 mM $H_2PO_4^-$ and 40 mM HCO_3^- (referred to as saline). Bovine serum albumin (BSA, Cohn fraction V (SERVA, Heidelberg, FRG)) and combinations of BSA + gamma-globulin (SERVA) or BSA + glucose were added to the saline in various concentrations.

Red blood cells were obtained from fresh defibrinated bovine blood. The blood was collected once a week by jugular vein puncture and defibrinated by stirring for 10 min. with a blender inserted into the blood-collecting jar. Upon arrival in the laboratory the cells and serum were separated in a clinical centrifuge at 2500 rpm for 20 min. Before use, the remaining RBC mass was washed with 0.9% NaCl in a ratio of 1:2 in order to remove remaining serum. To obtain the required diet, a known volume of washed RBC was combined with an equal volume of the artificial solution (50/50% = v/v). Finally, adenosine triphosphate (ATP) was added to each diet at a concentration of 10^{-3} M to serve as a feeding stimulus (Galun & Margalit, 1969). All diets were prepared under aseptic conditions.

The control diet consisted of equal volumes of washed RBC and fresh serum. The diets were 1-7 days old when they were offered to the flies and were stored at 4°C. Details of physical parameters and food-uptake and primary excretion have been described in a previous paper (Takken, 1980).

Results

The performance of control groups throughout the year exhibited high variability (Table I), the fecundity of control females generally decreasing with successive experiments. Because of this variability, results of semi-artificial diets will be expressed as the percentage of the results of the corresponding controls.

The performance of female *G. p. palpalis* over a 50-day period, fed on semi-artificial diets (RBC + serum substitutes), is summarized in Table II.

Survival

Survival appeared to be influenced by serum albumin in two ways. When no albumin was present a fairly high mortality occurred, especially in young flies. When albumin was present at the same concentration as in standard bovine blood (i.e. 4.5 g%), the survival was similar or even better than that of the controls. However, an increase in albumin above 4.5 g% increased the mortality. When 13.5 g% albumin was present (3x the standard blood value), only a few flies survived to the end of a 50-day period. The presence of 1 g% gamma-globulin or 0.07 g% glucose (both standard blood values) together with 4.5 g% BSA did not seem to influence the survival. Except for one case (saline) the survival during the productive period was similar over the total experimental period.

Productivity and Fecundity

The productivity is defined in this paper as the number of puparia produced per initial female over the total experimental period. It is influenced by fecundity and survival. Increasing levels of serum albumin in the diet improved productivity, but above 6.0 g% BSA it began to decline as result of increased mortality (Table II). The number of puparia produced per producing female per day (pppf) was chosen as a method to express fecundity independently of mortality. Under our rearing conditions a female is considered productive on day 18, the average day for the first larviposition. As a mature female of *G. p. palpalis*

Table I. Survival and reproductive performance of female *G. p. palpalis* fed on control diet (RBC + serum) for 50-day period.

control no.	date of emergence	no. of ♀♀	% survival		productivity[2]	fecundity[3]	puparial weight ± s.e. (mg)	
			exp. period (day 0-50)	productive[1] period (day 18-50)				
1	7 III 78	60	75	76	2.98	0.103	28.21	0.26
2	4 IV 78	48	63	75	2.10	0.073	26.75	0.42
3	2 V 78	60	77	78	2.35	0.083	26.19	0.31
4	20 VI 78	60	83	83	2.88	0.094	27.18	0.35
5	15 VIII 78	60	70	82	2.20	0.088	27.13	0.30
6	7 IX 78	59	66	74	1.63	0.064	27.61	0.34

1) on the average, a female produces her first larva on day 18 and is hence considered productive.

2) cumulative number of puparia per initial female.

3) viable larvae per female per day during reproductive period. Assuming that, on the average, a mature female larviposits every 9.5 day following larviposition, the expected figure is 0.105.

Table II. The survival and reproductive performance of female *G. p. palpalis* fed on different diets during a 50-day period.

diet (per 100 ml saline)	corresponding control[1]	no. of ♀♀	relative survival[1]		rel. productivity[1]	rel. fecundity[1]	puparial weight ± s.e. (mg)	
			exp. period	prod. period				
RBC + saline	2	61	69	92	4.8	7.9	13.94	0.9 *
RBC + saline (4.5 g BSA)	1,2	119	106.5	107.5	77.5	82.9	22.64	0.2 *
RBC + saline (6.0 g BSA)	6	58	89.4	92.7	79.1	78.4	24.42	0.5 *
RBC + saline (9.0 g BSA)	3,4,5	156	78.2	79	73.3	82.4	26.00	0.2 *
RBC + saline (13.5 g BSA)	4	39	39.8	54	60.4	101.1	27.19	0.5 *
RBC + saline (4.5 g BSA + 1 g γ-globulin)	2	57	128.6	113.6	101	100.7	22.31	0.3 *
RBC + saline (6.0 g BSA + 1 g γ-globulin)	6	59	89	85.9	79	77.2	24.61	0.1 *
RBC + saline (4.5 g BSA + 0.07 g glucose)	2	59	112.7	112.0	87.1	100.7	22.73	0.4 *

1) Results expressed as percentage of corresponding controls in Table I.

* Puparial weights significantly different from corresponding control ($P < 0.01$).

may larviposit every 9.5 days (Van der Vloedt, 1974) the maximum possible pppf figure is 0.105. The total absence of BSA in the diet resulted in a pppf figure of less than 8% of the control (Table II). In addition an exceptionally large number of eggs, embryos and non-viable larvae were deposited, especially as females grew older (Table III). Only 6 puparia were produced by 61 females and all puparia were non-viable. However, the addition of BSA to the diet improved the fecundity. It was 20% less than that of the control for diets with 4.5, 6.0 and 9.0 g% BSA, but with 13.5 g% BSA the fecundity was similar to the control. The combination of 1 g% gamma-globulin + 4.5 g% BSA or 0.07 g% glucose + 4.5 g% BSA resulted in a much better fecundity than 4.5 g% BSA alone. However, when the BSA concentration was increased to 6.0 g%, the addition of gamma-globulin did not improve fecundity.

The percentage of females which produced viable and non-viable progeny per age group period (AGP) of 10 days is expressed for each diet separately in Table III as means of all replicates. It is apparent that the maximum possible fecundity (all females producing viable offspring over four AGP's) was never achieved. On a diet without BSA, no puparia were produced during the 2nd and 3rd AGP. Only very few puparia were produced during the 4th and 5th AGP. The percentage of non-viable progeny, however, was consistently higher than that of the control in the 3rd, 4th and 5th AGP. The addition of BSA to the diet resulted in a production of viable and non-viable progeny which was very similar to that of the control, except for a diet with 13.5 g% BSA, which gave a higher percentage of viable progeny up to the 5th AGP than in the control. With 6.0 g% BSA a low output of viable progeny was obtained. This may have been caused by a relatively poor performance of the mother colony at the time of the experiment, since the corresponding control also had a very low fecundity. The addition of 1 g% gamma-globulin or 0.07 g% glucose to the albumin diets did not result in appreciable differences as compared to the control or the diets containing albumin only.

Table III. Percentage of females producing viable and non-viable[1)] progeny during successive age-group-periods (AGP).

diet (per 100 ml saline)	no. of ♀♀	2nd AGP (day 11-20)		3rd AGP (day 21-30)		4th AGP (day 31-40)		5th AGP (day 41-50)	
		viable progeny	n-viable progeny	viable progeny	n-viable progeny	viable progeny	n-viable progeny	viable progeny	n-viable progeny
RBC + serum (control)	287	30.4	10.3	81.1	22.1	74.5	13.4	77.2	20.1
RBC + saline	61	0	4.7	0	30.6	11.8	29.4	6.7	36.7
RBC + saline (4.5 g BSA)	119	27.4	12.7	76.6	20.6	61.6	18.2	71.4	32.8
RBC + saline (6.0 g BSA)	58	0	17.6	31.4	13.7	64.4	15.6	67.7	18.9
RBC + saline (9.0 g BSA)	156	26.5	17.6	66.1	16.2	89.0	18.3	78.5	17.2
RBC + saline (13.5 g BSA)	39	50.5	13.4	89.0	7.4	82.0	18.0	73.0	24.9
RBC + saline (4.5 g BSA + 1 g γ-globulin)	56	26.0	11.2	78.0	9.3	56.0	15.3	75.0	20.9
RBC + saline (6.0 g BSA + 1 g γ-globulin)	59	0	14.5	44.2	19.2	50.0	4.2	65.9	18.2
RBC + saline (4.5 g BSA + 0.07 g glucose)	59	22.0	18.0	78.0	12.2	72.0	12.7	57.0	25.0

1) % non-viable progeny: % ♀♀ producing eggs, embryos or non-viable larvae.

Puparial weight

The weight of the progeny was correlated with the concentration of BSA in the diet. The presence of 1 g% gamma-globulin or 0.07 g% glucose together with BSA did not have any effect (Table II). The relationship between puparial weight and BSA concentration in the diet is illustrated in Fig. 1. Data were taken from Table II. A linear relationship existed, described by the equation $y = a + \frac{b}{x}$ (y = puparial weight, x = BSA conc. (g%)). The co-efficient of correlation of 0.993 was significant ($P < 0.01$).

Discussion

In a study to evaluate the nutritional superiority of defibrinated porcine blood to bovine blood in the *in vitro* feeding of *G. m. morsitans*, Langley *et al.* (1978) have shown that on a diet without erythrocytes (e.g. saline or serum only) flies failed to reproduce, thus emphasizing the importance of corpuscles in the diet. When erythrocytes were suspended in saline, however, *G. m. morsitans* reproduced as was expected but the offspring were small. In the present study, *G. p. palpalis* failed to reproduce when they were fed on a suspension of erythrocytes. This cannot have been caused by a lower intake of food since the ingestion rates of flies fed on whole blood (RBC + serum) or on semi-artificial blood (RBC + serum-substitute) were similar (Takken, 1980). Although erythrocytes provide the bulk of protein in the diet of tsetse flies (> 90%), they apparently lack vital elements which are necessary for successful reproduction in *G. p. palpalis*. In the absence of serum, RBC's may not be properly digested, since the secretion of proteolytic enzymes is stimulated by serum rather than by RBC (Langley, 1966; Gooding, 1974, 1977). Indeed, in the presence of BSA or BSA + gamma-globulin, the flies reproduced normally and did not show any unusually large number of unsuccessful cycles, in contrast to the case with RBC suspended in saline only. It is, however, noteworthy that the fecundity was positively correlated with the concentration of BSA in the diet. A similar effect of serum albumin on fecundity was demonstrated for *G. m. morsitans* by Moloo

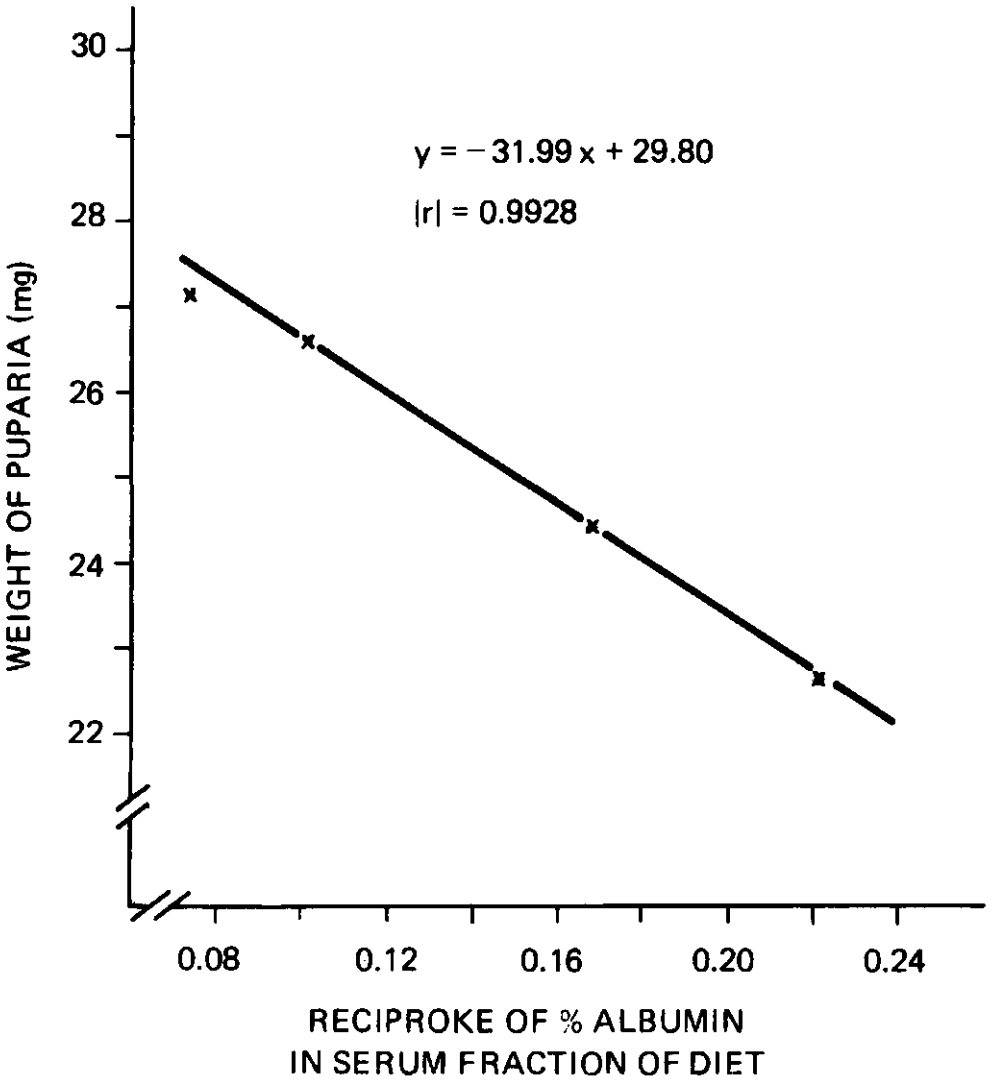

Figure 1. Regression of puparial weight (y) on serum albumin concentration (x) in the diet of *G. p. palpalis*.

& Pimley (1978). The addition of serum albumin to defibrinated blood significantly increased the fecundity of the females. However, these authors added serum proteins to defibrinated blood, whereas in the present study, serum proteins were added to washed corpuscles. These results indicate that the serum proteins may play a more important role in the reproductive physiology of tsetse flies than was previously thought.

Studies with labelled amino-acids and glucose have provided information on the metabolic pathways and incorporation of these substances in the female tsetse fly (McCabe & Bursell, 1975; Moloo, 1976a, 1976b; Moloo & Pimley, 1978), but no such studies have been reported on the digestive products of serum proteins or RBC. Recently, the absorption of serum proteins by *G. m. morsitans* was investigated by Nogge & Gianetti (1979). It was found that complete serum-globulins cannot pass through the peritrophic membrane, but fragments of globulins and albumin might do so. Up to 0.035% of the albumin content of a blood meal was recovered from the haemolymph. Serum albumins can thus be absorbed without enzymatic digestion. The nitrogenous content of the urine of tsetse flies consists mainly of uric acid (Bursell, 1965). Only very small amounts of free and protein-bound amino acids occur in the urine, presumably all excretory products (Balogun, 1974). As, however, only a very small fraction of complete albumin was recovered from the haemolymph, the albumin must be, at least partially, digested in the midgut. Except for the carrier function (Nogge & Gianetti, 1979) and the evidence that albumin is required for normal reproduction, a further explanation of the role of this protein in the tsetse fly can only be hypothetical.

In the presence of 4.5 g% BSA, the addition of gamma-globulin or glucose resulted in higher fecundities. Apparently either gamma-globulin or glucose also contribute directly to optimal reproduction or perhaps enhance the effects of BSA. With an increased level of BSA (6.0 g% BSA + 1 g% γ-globulin) the effect of gamma-globulin may have been masked or was affected by the same factors which caused a poor performance of the control flies. However, gamma-globulin or glucose had no effect on the offspring size. This is in agreement with results from the study

on *G. m. morsitans* of Langley *et al.* (1978). Moloo & Pimley (1978) reported that the addition of BSA to defibrinated bovine blood positively influenced the offspring size of *G. m. morsitans*. This agrees well with the present findings for *G. p. palpalis*, where a curvi-linear relationship exists. The viscosity, caused by a high level of BSA (13.5 g%), might have impaired a proper digestion resulting in mortality. From the results, the combination of RBC + saline (containing 9.0 g% BSA) seems an alternative diet for *G. p. palpalis*. The offspring are only slightly smaller and the fecundity is only 15% less than that of the control group. The fecundity might possibly be increased by the addition of 0.07 g% glucose. The results suggest, however, that the presence of serum albumin in the diet of *G. p. palpalis* is essential for a normal reproductive physiology and production of viable offspring.

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The effect of an albumin deficient diet on the reproductive performance of *Glossina palpalis palpalis* females (Diptera: Glossinidae)

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Abstract

Flies were fed on a mixture of washed bovine erythrocytes and artificial sera, which had previously been lyophilized. It was found that egg-maturation and ovulation were seriously impaired on a serum albumin deficient diet, and egg follicles were observed to be retained and lysed in the ovaries. Flies fed on a diet containing serum albumin ovulated, but the fecundity was low and puparia were significantly smaller than that of control flies; 42 per cent of the eggs and embryos were extruded from the uterus. The effects of dietary substances on egg-maturation, ovulation and successful egg-hatching are discussed.

Introduction

Tsetse flies reproduce by adenotrophic viviparity. In mated females the first egg is ovulated from the right ovary and passed into the uterus about 7 to 9 days after emergence; if the female is inseminated, the fertilized egg develops in the uterus into a fully developed third instar larva which is deposited on the ground to pupate. The developing larva is nourished from the 'milk' gland and consequently derives all its nutrients from the mother insect (Roubaud, 1909). Once the first larva has been produced, the reproduction cycle continues regularly and one larva is produced every 9 to 10 days (Mellanby, 1937; Saunders, 1972; Denlinger and Ma, 1974). The close parent-offspring relationship suggests that adequate nutrition of the parent insect is necessary for a regular reproductive cycle. Mellanby (1937), studying the reproduction of *Glossina palpalis*, observed that insufficient blood uptake would lead to frequent abortions and even to a complete interruption of the normal reproductive cycle. In the course of a recent study to reveal the nutritional requirements of *G. p. palpalis* (Rob.-Desv.) it was found that certain diets seriously affected the fecundity and flies would not produce viable larvae (Takken, 1980b). These effects, however, were not due to insufficient food uptake. Serum albumin proved to be essential for normal larval production and the absence of this protein in the diet resulted in unproductive flies. In order to elucidate this diet-induced sterility, an examination was conducted of the ovaries of flies fed on semi-artificial diets in which serum was replaced by artificial feeding solutions. Results of this study are reported here and discussed in the light of future developments of artificial feeding for tsetse flies.

Materials and Methods

Newly emerged flies (0-24 h) were obtained from a colony of

G. p. palpalis which had been maintained on defibrinated, lyophilized bovine blood for 15 months (Wetzel, in press)*.

Female flies were divided into groups of 60 flies, four cages with 15 females each. Each cage served as a replicated within the group. The females were mated on day 3 after eclosion with 7-day old males and were separated 2-3 days thereafter. The flies were offered food 6 times a week. Mortality and fecundity were recorded daily except Sundays. Puparia were weighed individually when they were 0-24 h old except on Mondays, when those produced since Saturday were also included. The maintenance and feeding techniques have been described by Wetzel and Luger (1978).

An additional 135 females, which were to be dissected, were likewise treated and divided into three groups of 45 females, 15 females per cage. At specific time intervals the reproductive organs of these flies were dissected in 0.9% saline, fixed in Carnoy's solution and mounted. Ovarian nomenclature was used according to Saunders (1960).

In contrast to the previous experiments in which fresh blood was used (Takken, 1980b), flies were here fed on lyophilized diets, which had been prepared as follows: bovine blood was collected by venajugular puncture under aseptic conditions and defibrinated immediately after collection. Upon arrival at the laboratory, the blood was spun in a clinical centrifuge at 3500 rpm for 20 min.. The serum-fraction was drawn off and the remaining cell-fraction was washed with a solution of 0.89% NaCl in double distilled water at a ratio of one part cells to three parts saline. The cell suspension was spun at 3500 rpm for 20 min. and the thus processed blood cells were used throughout the experiment and will be referred to as 'RBC'. Two artificial serum-solutions were prepared. Solution no. 1 consisted of a sterile, isotonic saline containing 140 mM Na^+, 5 mM K^+, 100 mM CL^-, 5 mM $H_2PO_4^-$, 40 mM HCO_3^- and 0.07% glucose (referred to as 'saline'). Solution no. 2 was almost identical to this saline, ex-

* The *in vitro* colony of *G. p. palpalis* at Seibersdorf had been maintained on defibrinated blood since March 1976 (Wetzel and Luger, 1978) and was transferred to a diet of lyophilized blood exclusively in August 1978.

cept that it also contained 6% bovine serum albumin (BSA (SERVA, Heidelberg, FRG)) and will be referred to as 'saline (6% BSA)'. Osmolarity and pH of the solutions were identical to those of bovine serum (Takken, 1980a).

Three different diets were prepared by making combinations of RBC + fresh serum, RBC + saline and RBC + saline (6% BSA) in a ratio of 4:6 by volume. These diets were deepfrozen at -37°C and lyophilized as described by Wetzel (in press). The lyophilized diets were stored under vacuum in the dark at room temperature until required. To prepare the diets for feeding, the lyophilized material was dissolved with a magnetic stirrer in double distilled water in the volume, as had been removed during lyophilization, minus 10% in order to obtain the same osmolarity as that of defibrinated fresh blood. Each time a sample was prepared, care was taken that the osmolarity and pH of the diet were similar to those of fresh blood. Finally, 10^{-3} M adenosine-triphosphate (ATP) was added as a feeding stimulus (Galun and Margalit, 1969). All handling took place under aseptic conditions. Reconstituted diets were never older than 62 h when they were offered to the flies. The diet made up of lyophilized RBC + serum is considered as the control diet throughout this experiment.

Results

The performance of the flies (Table I) shows that there was not much difference in survival for the control flies and those fed RBC + saline (6% BSA) up to day 30, though there was a large variability between individual cages. In general, the mortality was high and exceptionally high for the group fed on RBC + saline, which was terminated after 30 days since only 35 per cent were alive at that time. The fecundity index of the control flies was 0.084 and significantly higher (t-test, $P < 0.001$) than that of both groups on experimental diets. On RBC + saline not one single egg or larva was produced up to day 30, while on RBC + saline (6% BSA) 25 per cent of the females produced regularly a viable larva (control: 59 per cent). However, regarding total offspring (that is eggs, embryos and larvae), it appears

Table I. Performance of fertilized female *G. p. palpalis* fed on different diets over a 50-day period.

d i e t	no.of ♀♀	% survival at days: 10	20	30	40	50	fecun-* dity	total offspring: eggs + embryos	non-viable larvae	viable larvae	puparial weight ± se (mg)
RBC + serum (control)	60	92	88	78	67	57	0.084	28	18	122	25.73 0.43
RBC + saline	60	67	53	35	x	x	0.000	-	-	-	-
RBC + saline (6% BSA)	59	88	86	80	75	64	0.034	54	23	52	23.03 0.58

x discontinued after 30 days because of high mortality.

* viable larvae per female per day during reproductive period. Assuming that on the average, a mature female larviposits every 9.5 days following larviposition, the maximal expected figure is 0.105.

that the flies fed on a serum-supplemented diet which included albumin produced an unusual large number of eggs, embryos and non-viable larvae (60 per cent of total) throughout the experimental period. Of the total offspring in the control group, 29 per cent consisted of 'abortion'. The puparia of the RBC + saline (6% BSA) fed flies were significantly smaller (*t*-test, $P < 0.001$) than those of the control.

Dissections of 10-, 20- and 30-day old females showed that almost all females were inseminated (Table II), but the group fed RBC + saline did not ovulate. Only in one case an egg was found in the uterus. Follicles were found retained and lysed in the ovaries, the remnants obstructing the common oviduct. Details of the length of the follicles will be published elsewhere (Takken, in prep.) but it should be mentioned here that throughout the experimental period follicle development of flies fed on experimental diets was slower than that of control flies.

Examples of normal and abnormal developments in the different experimental groups are shown in Figures 1-6. Figures 1a and 1b show the reproductive system of 10-d old control females, 1a has recently ovulated, while in 1b the first egg (A_1) is on its way to the uterus. Fig. 2 shows the situation in a 10-d old female, fed on RBC + saline. The follicle in ovariole A is in degeneration and will not be ovulated. C_1 looks normal so far. The uterus was empty. A 20-d old female, fed RBC + saline (6% BSA), in which C_1 may be ovulated shortly, while B_1 is already in an advanced stage of development, is shown in Fig. 3. This is very unusual and indicates that A_1 was upheld unusually long. The uterus was empty. Fig. 4 shows the reproductive organs of another 20-d old female, fed RBC + saline. A_1 is completely degenerated and the remnants block the common oviduct. C_1 has developed but is in a less advanced stage than in the other two groups. In Fig. 5 the reproductive organs of a 30-d old control female are shown. There is a second larval instar in the uterus (resulting from follicle B_1) while D_1 is in an advanced stage of development. A_2 can be seen clearly. Figures 6a and 6b show the situation in 30-d old flies fed on RBC + saline. Both situations were never observed in the controls, but were found in all experimental females.

Table II. State of the reproductive organs of female *G. p. palpalis* fed on different diets. n - number of females; sp. - females with sperm-containing spermatheca; ut - uterine content; fol. - the most advanced follicle of females, whose uterus was found empty.

female age (days)	d i e t	n	sp.	ut.	fol.	remarks
10	RBC + serum	9	8	7xegg	2xA_1	
10	RBC + saline	10	7	1xegg	9xA_1*	* occasionally A_1 in process of degeneration.
10	RBC + saline(6% BSA)	8	8	6xegg	2xA_1	
20	RBC + serum	8	8	3xegg 3xL_2 1xL_3	1xA_1*	* blockage
20	RBC + saline	8	8	-	2xA_1 6xC_1	
20	RBC + saline(6% BSA)	11	10	1xegg 1xL_1 1xL_2 1xpupa	1xC_1 3xB_1 2 abnormal	
30	RBC + serum	9	9	5xegg 3xL_1	1xD_1	* remnants of degenerated follicles obstruct the common oviduct.
30	RBC + saline	11	11	-	*	
30	RBC + saline(6% BSA)	8	8	2xegg 1xL_2 1xpupa	1xA_1** 2xB_1 1xD_1	** blockage

Discussion

It was earlier reported (Takken, 1980b) that flies fed on a diet of RBC + saline frequently aborted and did not produce viable offspring. When fed on RBC + saline (6% BSA) the fecundity index was 0.066 and the puparia were slightly undersized. Langley *et al.* (1978) did not find an impaired fecundity when feeding *G. m. morsitans* on a diet of fresh, washed RBC + saline, though they observed a greater offspring size when such diets were supplemented with serum albumin. However, the final results could have been influenced by a slight modification of the saline, which in their case contained calcium and magnesium. These diets were not lyophilized. Feeding lyophilized blood to *G. p. palpalis* does not alter the performance when results are compared with those obtained from flies fed on fresh blood (Wetzel, in press). This is also suggested when the results of the present experiment are compared with earlier results when flies were fed fresh RBC + serum (Takken, 1980b). What then causes the complete unproductivity of flies fed lyophilized RBC + saline and the low fecundity of those fed lyophilized RBC + saline (6% BSA)? In the present experiment whole blood and cells were spun at 3500 rpm and this may have removed fractions which remain mixed with the erythrocytes when they are spun at 2500 rpm as was the case in the previous study (Takken, 1980b). In a recent experiment (author, unpublished) it was found that addition of 2.5 mM CA^{++} and 1.5 mM Mg^{++} to the saline (6% BSA) resulted in a normal fecundity and since these minerals are present in minimal quantities they may well have been removed during centrifugation at 3500 rpm. As mentioned before, Langley *et al.* (1978) routinely added these minerals to their diets and possibly therefore never observed a reduced fecundity. It is, however, of interest that the fecundity of *G. p. palpalis* is albumin dependent and that the actual process of follicle development can be inhibited because of lack of nutritional substances (i.e. serum albumin).

Several species of mosquitoes can be kept alive for several months on sugar solutions alone, but they need protein to mature their eggs (Lea *et al.*, 1956). *Anopheles elutus* failed to oviposit when fed washed donkey erythrocytes (Yoeli and Mer, 1938)

and *Aedes aegypti* had the highest egg production on a mixture of sheep erythrocytes with either egg- or human-albumin (Greenberg, 1951); however, it did not reproduce on washed erythrocytes only.

Virgin tsetse flies develop the first oocyte at the same rate as mated flies. By day 8-9, when normally the first egg is ovulated, virgin flies retain their egg in the ovary and ultimately will resorb it, only leaving the chorion (Odhiambo, 1971). Occasionally, however, mature eggs have been found in the uterus of 5-7 weeks old virgin females, possibly pushed out of the ovaries by lack of space (Mellanby, 1937; Jaenson, 1979). Mating (and not spermatophore building, sperm-transfer and -uptake in the spermathecae) is required for ovulation in tsetse flies (Saunders and Dodd, 1972; Chaudhury and Dhadialla, 1976). Females remember the mating experience and a neuro-endocrine control of ovulation (ovulation hormone) was demonstrated (Foster, 1974; Ejezie and Davey, 1974), though it is not yet fully understood at what level it acts (Tobe and Langley, 1978). In this experiment, eggs of inseminated females were found to be retained and lysed in the ovaries of flies fed an albumin-deficient diet, leading to the hypothesis that either the follicles never matured or that the ovulation control system had been disturbed. Since oocyte growth takes place in flies fed RBC + saline, vitellogenin proteins are apparently produced and incorporated in the oocyte. Vitellogenesis in *Glossina*, unlike in most insects (Engelmann, 1970), takes place in the follicular epithelium (Huebner *et al.*, 1975) and therefore may be independent of juvenile hormone (JH) control (Ejezie and Davey, 1974). Consequently the disturbance of follicle development is unlikely to be caused by a malfunctioning at this level of endocrine control.

Nutrition plays a role in the growth of oocytes of *Glossina* (Saunders, 1961; Foster and Allingham, 1972), but the reported studies all deal with the actual blood-uptake and timing of feeding to oocyte growth of insects fed on live hosts (c.q. normal blood). In this study, however, we are dealing with a deficient diet (lacking serum albumin), resulting in a disturbance of the follicle development. Tobe and Langley (1978) mention

that the availibility of nutrients in the haemolymph may regulate in part the rate of vitellogenesis in the growing oocyte. In this aspect Nogge and Gianetti's (1979) observation that complete serum albumin can pass the midgut wall undigested into the haemolymph of *G. m. morsitans* may throw some light on the role of serum albumin in *Glossina*'s diet. Lack of essential nutrients may well be responsible for the observed effects in the present study. However, the content of the ovariole has not been studied in detail and there is no evidence that it consisted of a mature and chorionated egg. On the contrary, Figures 2 and 4 suggest that the egg follicles never matured. In fact they degenerated already at an early stage in adult life, unlike in females that were kept virgin for 20 days and, after mating, occasionally produced a viable larva originating from the first oocyte (Ejezie, 1976). The timing and sequence of oocyte growth in females, fed on RBC + saline, is apparently not disturbed, since at day 30 the third and fourth oocytes in line (B_1 and D_1) are developing as in control flies, however with A_1 and C_1 degenerated and obstructing the common oviduct (Figures 6a, 6b). If in mated females the oocytes develop into maturity without being ovulated, then the ovulation control system must have been disturbed, either at the MNSC level or at the level of hormone release (Chaudhury and Dhadialla, 1976). Denlinger *et al.* (1978) suggested that cyclic-AMP might be an additional trigger for ovulation in that it stimulated the release of the ovulation hormone from the brain. Obviously many steps are involved in ovulation and it is quite likely that inadequate nutrition results in disbalance.

Clearly then, one would suspect that on diets, lacking serum albumin, either the neuro-endocrine control of ovulation is disturbed in flies fed on RBC + saline, or that the oocytes never mature and hence cannot be ovulated.

The diets that included serum albumin are inferior to the control diets because of the high abortion rate and low fecundity obtained. Although the amount and composition of the milk-gland secretion might be partially responsible for an inadequate nutrition of the growing intra-uterine larva (Cmelik *et al.*, 1969; Moloo, 1976; Langley and Pimley, 1979), this seems unlike-

ly to be the case here, since 70 per cent of the abortions consisted of eggs or embryos. Also, the larval abortion rate was not significantly higher than that of the control flies. Fertilization had taken place but some eggs may not have been able to undergo successful embryonic development or were too weak to hatch. It is tempting to relate the observed effects to the removal of serum substances which had not been removed in the previous experiments in which these effects did not occur with diets containing serum albumin (Takken, 1980b). The observation that the presence of Ca^{++} and Mg^{++} can prevent a low fecundity or high abortion rate, suggests that these minerals might be incorporated in the oocytes, and in part are responsible for successful embryonic development or hatching of the intra-uterine egg. Lea *et al.* (1956) mention that minerals may be a factor of egg-formation in mosquitoes, but they do not give further details.

From the results one may conclude that serum albumin is an essential nutritional element in the diet of female *G. p. palpalis* and influences the oocyte growth and subsequently also ovulation. Thorough washing of erythrocytes resulted in biochemical changes which require the addition of saline containing serum albumin and possibly also Ca^{++} and Mg^{++} for reproduction in *G. p. palpalis*. The role of the last two elements needs further investigation.

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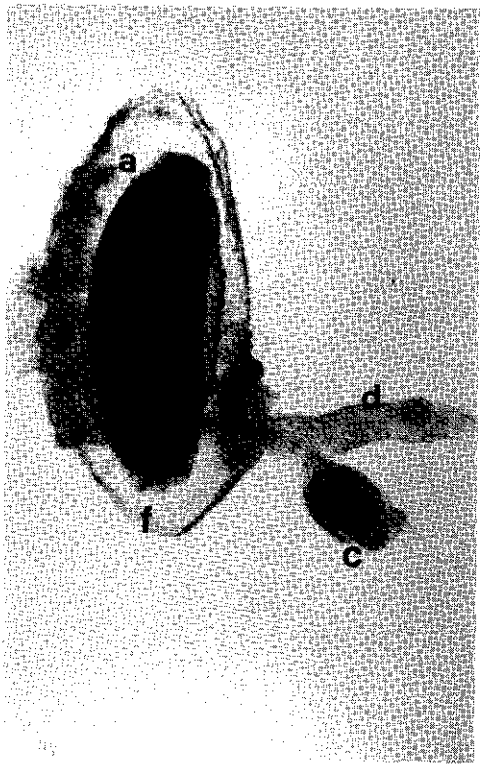
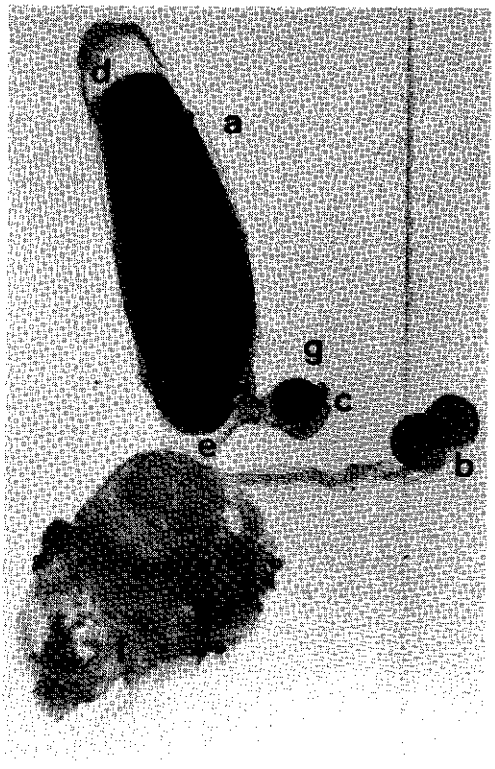
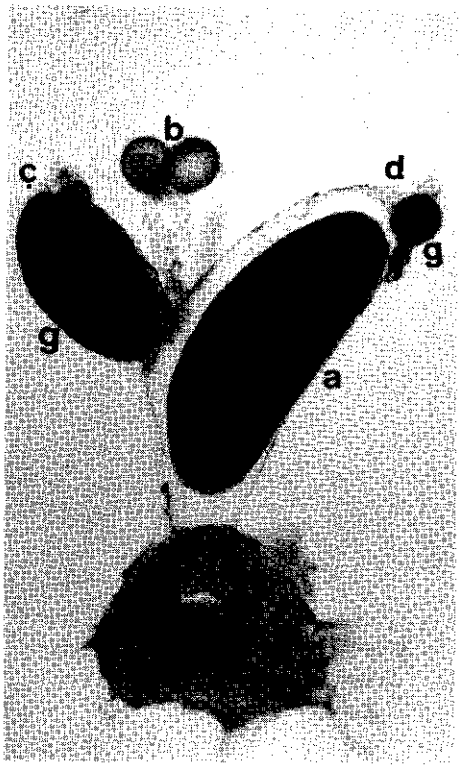




Figures 1a, 1b and 2. Reproductive organs of 10-day old females of *G. p. palpalis*.
1a, 1b - flies fed on RBC + serum; 2 - a fly fed RBC + saline.
a) egg; b) spermatheca; c) right ovary; d) left ovary; e) common oviduct; f) uterus; g) developing follicle(s).



Figures 3 and 4. Reproductive organs of 20-day old females of *G. p. palpalis*.

3 - a female fed on RBC + saline (6% BSA);

4 - a female fed on RBC + saline.

a) fully developed egg;

b) spermatheca;

c) right ovary;

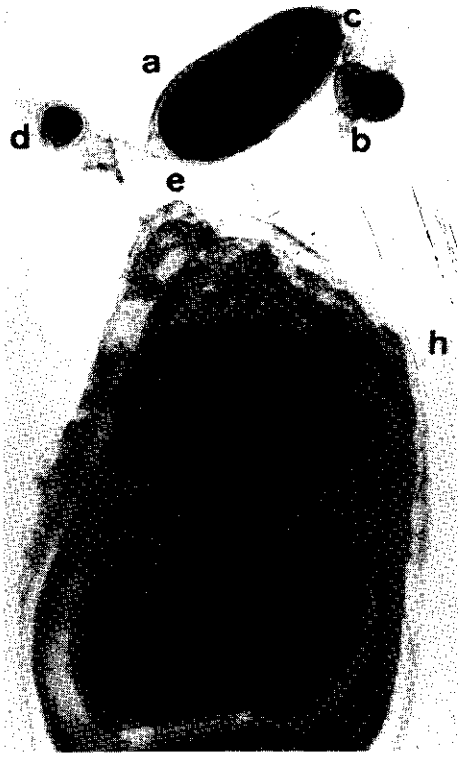
d) left ovary;

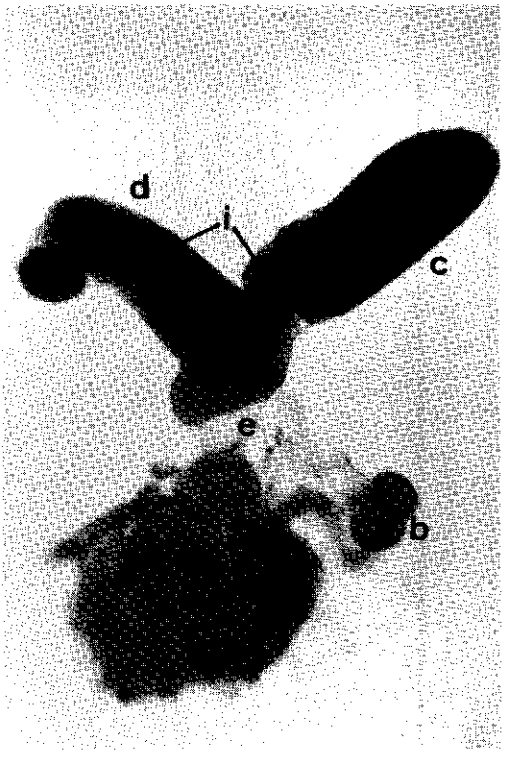
e) common oviduct;

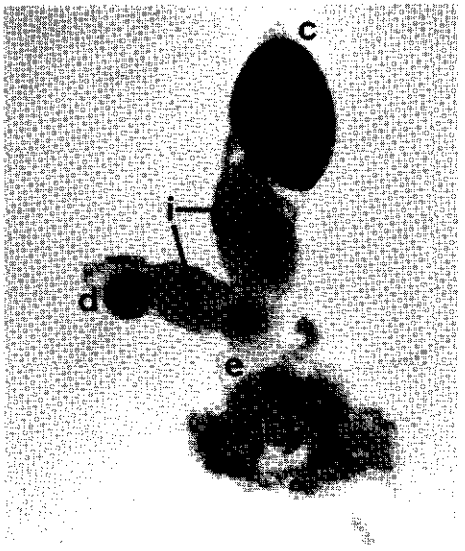
f) uterus;

g) developing follicle(s);

h) remnants of lysed egg.







Figures 5 and 6. Reproductive organs of 30-day old females of *G. p. palpalis*.
5 - female fed on RBC + serum; 6a, 6b - females fed on RBC + saline.
a) developing egg; b) spermatheca; c) right ovary; d) left ovary; e) common oviduct; f) uterus; g) developing follicle(s); h) L3; i) partially lysed eggs.

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Dynamics of follicle development and lipid storage in the tsetse fly, *Glossina palpalis palpalis* (Diptera: Glossinidae), fed on serum albumin deficient diets

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Abstract

Females of *Glossina p. palpalis* were fed on a mixture of bovine erythrocytes and a serum-substitute, that previously had been lyophilized, for a period of 30 days. The rate of ovarian development and lipid incorporation and the fatty acid composition of whole flies were examined at five day intervals. Results showed that in flies, fed serum albumin deficient diets, ovarian development was seriously impaired and ovulation did not take place. Flies which were fed on a serum albumin containing diet ovulated and produced larvae, though at a lower rate than control flies. Compared with controls, lipid incorporation was reduced in experimental flies, but more in the albumin deficient group than in the albumin containing group. The diet composition had no marked influence on the relative composition of fatty acids in any one group. It is concluded that, though an essential nutritional element for cow blood fed *G. p. palpalis*, serum albumin was not the only missing essential factor in the diets that were used in this study.

Introduction

Reproduction in tsetse flies (*Glossina* spp.) takes place by way of adenotrophic viviparity. Once the first egg has descended into the uterus, all reproductive activities are precisely synchronized and at 25°C one larva is produced every 9-10 days. The growing intra-uterine larva is fed from the female parent's 'milk'gland, and undergoes an exponential growth until it reaches maturity (Denlinger and Ma, 1974; Langley and Pimley, 1975). In early pregnancy, the female stores food reserves in her fat body, mainly as lipid (Bursell *et al.*, 1974; Moloo, 1976). At a later stage of pregnancy these lipids are mobilized and transferred to the milk gland (Tobe *et al.*, 1973; Moloo, 1976; Langley and Pimley, 1979b). The size of the newly deposited larva of *Glossina* was found to be correlated with the amount of serum albumin in the diet (Langley *et al.*, 1978; Moloo and Pimley, 1978; Takken, 1980b) and in *Glossina palpalis palpalis* (Rob.-Desv.) serum albumin appeared to be essential for the functioning of the reproductive physiology of the females (Takken, 1980b and in press).

In serum albumin deficient diets, females failed to ovulate fully. From the results it was felt that the function of serum albumin was not well understood, and a study was initiated to examine the relationship between serum albumin and lipid storage, lipid quality and ovarian development during several pregnancy cycles of *G. p. palpalis*.

Material and Methods

Flies

Newly emerged flies (0-24 h) were obtained from a colony of *G. p. palpalis* which had been maintained on defibrinated bovine blood since March 1976 (Wetzel and Luger, 1978), and then transferred to a diet of defibrinated, lyophilized bovine blood exclusively in August 1978 (Wetzel, in press). At the time of the

experiment the colony had been maintained on lyophilized blood for 15 months.

Fly handling

Females were placed 15 flies per cage and subjected to one of three experimental diets. Care was taken to ensure that the flies in the three groups were approximately the same age (within 3 days) in each replicated experiment. The females were mated on day 3 of their adult stage with 7-day old males and separated 2-3 days later. The males were taken from the stock colony (see above). Mortality and fecundity were recorded daily except Sundays. The flies were offered food 6 times a week. Those which were subjected to lipid extraction or dissection were given a last opportunity to feed 24 h before being sacrificed. Maintenance and feeding techniques have been described by Wetzel and Luger (1978).

Experimental diets

Flies were fed on lyophilized diets, which had been prepared as follows: bovine blood was collected by venajugular puncture under aseptic conditions and defibrinated immediately after collection. Upon arrival at the laboratory, the blood was spun in a clinical centrifuge at 3500 rpm for 20 min.. The serum fraction was drawn off, and the remaining cell fraction was washed with a solution of 0.89% NaCl in double-distilled water (cells to saline 1:3). The cell suspension was then spun at 3500 rpm for 20 min., and the thus processed blood cells were used throughout the experiment (referred to as 'RBC'). Two artificial serum solutions were prepared. Solution no. 1 consisted of a sterile saline containing 140 mM Na^+, 5 mM K^+, 100 mM Cl^-, 5 mM $H_2PO_4^-$, 40 mM HCO_3^- and 0.07% glucose (referred to as 'saline'). Solution no. 2 was almost identical to this saline, except that it also contained 6% bovine serum albumin (BSA (SERVA, Heidelberg, FRG)) and will be referred to as 'saline (6% BSA)'. Osmolarity and pH of the solutions were identical to those of serum (Takken, 1980a).

Three different diets were prepared by making combinations

of RBC + fresh serum, RBC + saline and RBC + saline (6% BSA), all in a ratio of 4:6 by volume. These diets were deepfrozen at -37°C, and lyophilized as described by Wetzel (in press). The lyophilized diets were stored under vacuum in the dark at room temperature until required. To prepare the diets for feeding, the lyophilized material was dissolved with a magnetic stirrer in double distilled water. The volume of water added was 10% less than that removed during lyophilization, in order to obtain the same osmolarity as that of defibrinated fresh blood. Each time a sample was prepared, care was taken to ensure that the osmolarity and pH of the diet were similar to those of fresh blood. Finally, 10^{-3} M adenosine triphosphate (ATP) was added as a feeding stimulant (Galun and Margalit, 1969). All handling took place under aseptic conditions. Reconstituted diets were never older than 62 h when they were offered to the flies. The diet made up of lyophilized RBC + serum is considered as the control diet throughout this experiment.

Dissections

Mated females were dissected in 0.9% saline on day 10, 20 and 30 of their adult life. Spermathecae were checked for insemination, the uterus for the presence of an egg or larva, and ovarian development examined by measuring the length of the 2 largest follicles, using a calibrated ocular micrometer (Saunders, 1960).

Lipid extraction

Newly emerged females (0-3 h old) and females which had fasted for at least 24 h, were killed in ethyl acetate, weighed on a Mettler analytical balance, and stored at -37°C until used for lipid extraction. Extractions were performed on single flies, 10 females per treatment per age group. Flies were homogenized in chloroform-methanol (2:1) (Folch *et al.*, 1957). The crude extract was filtered, and the lipids separated by washing in chloroform-methanol-water (2:2:1.8) (Bligh and Dyer, 1959). The purified extract was transferred to a 4.5 ml, pre-weighed glass vial, and dried under a gentle stream of nitrogen to con-

stant weight. If not used immediately, lipids were stored at -37°C under nitrogen.

Column separation

Pooled lipid extracts of at least 10 females, aged 0, 10 or 20 days, were separated into neutral and polar fractions by column chromatography with silicagel (Unisil 100-200 mesh, CLARKSON CHEMICAL CO., Williamsport, Pa, USA), according to the method of Rouser *et al.* (1967).

Fatty acid analysis

Fatty acids from neutral and polar lipids of 10- and 20-day-old females were analysed, using techniques modified from Enser (1975). After saponification and acidification, the fatty acids were extracted in petroleum benzene, methylated with 12% BF_3 in methanol, and the composition determined by gas-liquid chromatography.

Results

On the average, eight to eleven females per treatment per age group were dissected, and the reproductive organs examined. With the exception of four flies (RBC + saline, 10 days: 3 females; RBC + saline (6% BSA), 20 days: 1 female) all females were inseminated. By day 10, all females had ovulated, except for the group fed RBC + saline, in which only one female had ovulated (Table I). By days 20 and 30, none of the flies in this last group had ovulated, and degenerating eggs were obstructing the common oviduct. Flies fed on RBC + serum, or RBC + saline (6% BSA) had almost all ovulated by days 20 and 30. The average lengths of the two largest egg follicles are given for those flies which all had the same ovarian configuration (Table I). At all life stages, the control flies were in a more advanced stage of ovarian development than flies of both experimental groups, though those fed RBC + saline (6% BSA) were more advanced than females fed RBC + saline. In 30-day-old females fed RBC + saline (6% BSA) the follicles were found to be in different stages of

Table I. Development of the reproductive organs of fertilized *G. p. palpalis* females fed on different diets. n - number of females; ov. - number of females that had ovulated; ovar. conf. - according to Saunders (1960); follicle length - of the two largest follicles, corresponding with nos. 1 and 2 in the previous column.

female age (days)	d i e t	n	ov.	most prominent ovar. conf.					follicle length mean ± sd. (µm)	
				D	C	A	B	%		
10	RBC + serum	9	7	3	1	4	2	(80)	789 235	145 25
10	RBC + saline	10	1	4	2	1	3	(90)	1320 350	189 75
10	RBC + saline (6% BSA)	8	6	3	1	4	2	(75)	603 247	136 85
20	RBC + serum	8	8	2	4	3	1	(83)	1180 284	438 96
20	RBC + saline	8	0	3	1	4	2	(63)	915 320	240 111
20	RBC + saline (6% BSA)	11	9	2	4	3	1	(67)	940 255	167 70
30	RBC + serum	9	9	1	3	2	4	(89)	1103 275	327 71
30	RBC + saline	11	0	blockage				(100)	782 332	322* 71
30	RBC + saline (6% BSA)	8	8	1	3	2	4	(38)	1120 290	254 90

* the two largest follicles were B_1 and D_1.

development and no ovarian configuration was represented by more than 38% of the flies (DCAB = 1324) (other ovarian configurations were 2431 (3x), 3142 (1x) and 4213 (1x; A_1 obstructing the common oviduct)). The group fed RBC + saline showed a remarkable homogeneity at this age: all eleven dissected females contained degenerating A_1 and C_1 follicles; B_1 was the most advanced egg follicle.

The change in whole-body lipid content over a 30-day period is shown in Figure 1. Except for days 10 and 15, the differences in total lipids between control flies and flies fed RBC + saline were not significant (*t*-test, $P > 0.05$). Comparing controls with flies fed RBC + saline (6% BSA), differences were not significant on days 5 and 15 ($P > 0.05$), but on all other sampling days, the differences were significant, controls having a higher lipid content than flies fed RBC + saline (6% BSA) on day 10, but a lower lipid content from day 20 onwards ($P < 0.05$).

At all stages of life, most of the lipids consisted of neutral lipids regardless of the diet (Table II). However, by day 10, the control flies had built up a higher lipid reserve than flies fed RBC + saline or RBC + saline (6% BSA). At this age, all groups had increased their polar-lipid contents, as compared to newly emerged females, but the group fed RBC + saline contained fewer polar lipids than both other groups. The lipid fractions of 20-day old females fed RBC + saline (6% BSA) were very similar to those of 10-day old controls.

There was not much difference in the relative fatty acid composition of neutral lipids extracted from females fed on the experimental and control diets (Table III). Similarly, there was no apparent difference with age: 10-day old females of *G. p. palpalis* had the same fatty acid composition of neutral lipids as 20-day old females. Palmitic acid, palmitoleic acid and oleic acid were the major fatty acids in the neutral fractions with palmitic acid the predominant and present in amounts 2-3 times that (by weight) of the two other fatty acids. However, the RBC + saline group tends to have relatively slightly elevated levels of stearic and oleic acids, and slightly less palmitic acid, than the other groups.

The polar lipids showed some differences in relative fatty

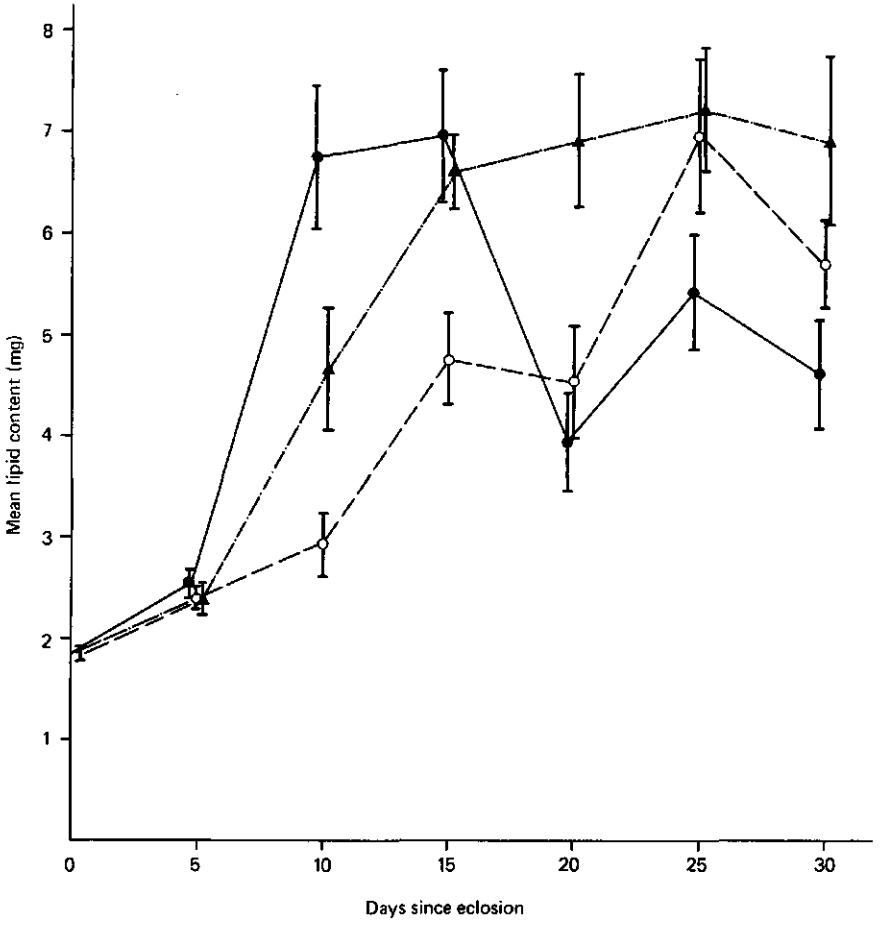

Figure 1.

Total lipid content (mg) in relation to age (d) of fertilized female *Glossina p. palpalis* fed on different diets since eclosion: ○ RBC + serum; ● RBC + saline; ▲ RBC + saline (6% BSA). Points are the means ± se of 10 flies. The arrow on the x-as indicates parturition among control flies.

Table II. Mean lipid content of fertilized female *G. p. palpalis* fed on different diets.

female age (days)	combined lipid fraction	mean lipid content per fly (mg)*		
		RBC + serum	RBC + saline	RBC + saline (6% BSA)
0	neutral	1.37		
	polar	0.50		
10	neutral	5.77	2.18	3.58
	polar	1.05	0.65	0.99
20	neutral	3.19	3.67	5.72
	polar	0.81	0.79	1.06

* values are the means of 10 flies.

acid composition, both between flies from different diets and at different ages (Table III). Polar lipids from 10-day old females fed on a control diet, or on RBC + saline (6% BSA), had a very similar fatty acid composition, but flies fed RBC + saline contained relatively more oleic and linoleic acid, and less palmitic and palmitoleic acid. In 20-day old females, the fatty acid composition of control and RBC + saline fed flies were similar, but the RBC + saline (6% BSA) fed flies had markedly less oleic acid and relatively more palmitoleic acid than either of the former. Except for a somewhat larger presence of oleic acid in 20-day old control females, the fatty acid composition of polar lipids was similar, in general, to that of 10-day old females. Palmitic, palmitoleic and oleic acids were also the major fatty acids of polar lipids in *G. p. palpalis*, but were present in different ratios than in neutral lipids: oleic acid was the predominant, in amounts 2 to over 3 times that (by weight) of palmitic acid, and 1.5 to almost 5 times that of palmitoleic acid.

Palmitic, palmitoleic and oleic acids together comprise

Table III. Fatty acid composition (% by weight of total fatty acids) of the whole-body neutral and polar lipids of fertilized 10- and 20-day old female *Glossina p. palpalis*, fed on different diets.

Age (days)	Fatty acid	Carbon no.	NEUTRAL LIPIDS (%)			POLAR LIPIDS (%)		
			RBC + serum	RBC + saline	RBC + saline(6% BSA)	RBC + serum	RBC + saline	RBC + saline(6% BSA)
10	Myristic	C_{14}	1.3	1.5	0.8	0.4	0.3	1.1
10	Palmitic	C_{16}	52.6	49.8	52.8	20.1	16.1	18.8
10	Palmitoleic	$C_{16:1}$	17.7	17.7	17.6	22.6	12.4	24.3
10	Stearic	C_{18}	3.6	4.5	4.0	5.0	4.9	3.6
10	Oleic	$C_{18:1}$	23.8	25.3	23.8	46.9	57.0	46.4
10	Linoleic	$C_{18:2}$	1.0	1.2	1.0	5.0	9.3	5.8
20	Myristic	C_{14}	1.7	1.4	1.4	0.8	0.5	0.6
20	Palmitic	C_{16}	52.2	51.2	52.8	17.9	17.6	18.0
20	Palmitoleic	$C_{16:1}$	17.8	16.6	18.6	21.8	20.7	26.4
20	Stearic	C_{18}	3.9	5.1	4.1	3.1	3.2	2.8
20	Oleic	$C_{18:1}$	23.3	25.2	22.4	50.0	52.7	43.5
20	Linoleic	$C_{18:2}$	1.1	0.5	0.7	6.4	5.3	8.7

over 90% of the neutral-lipid fatty acids (by weight) and over 85% of the polar-lipid fatty acids. Linoleic acid was present in relatively higher concentrations in the polar than in the neutral fractions. In all samples studied, decanoic acid, lauric acid, linolenic acid, and fatty acids belonging to the C_{19}-C_{22} group were not detected but may have been present in trace amounts.

Discussion

From the results it appears that the follicle development of *G. p. palpalis* is seriously impaired in flies fed diets which are deficient in bovine serum albumin. Artificial diets which are supplemented with albumin are particularly effective in overcoming the inhibition in young females. The recent observation that egg follicles of flies fed on albumin-deficient diets did not mature and were lysed inside the ovaries (Takken, in press), and the presently demonstrated inhibition of follicle development, cannot be the result of inadequate meal size or mating, since the experimental flies engorged to the same degree as controls and were inseminated (Takken, 1980a and in press). Moreover, meal size and insemination may not be critical factors for follicle development and maturation of the egg in *G. p. palpalis*. In newly emerged adults of *G. morsitans* the rate of development of the egg follicles increased significantly after a blood meal had been taken (Saunders, 1961). However, the size of the meal did not affect the rate of follicle growth, and there was also no threshold of bloodmeal size, below which ovarian development did not occur. Furthermore, follicle development in *Glossina* is independent of the mating act since virgin females develop the first oocyte at the same rate as mated females (Odhiambo, 1971). It is known, however, that eggs can only be ovulated when they are considered mature, i.e. when the chorion has been laid down (Tobe and Langley, 1978). It has been demonstrated that 11-12 d old virgin females of *G. m. morsitans* (which are past the normal ovulation time at d 9-10) can ovulate within 24 h after mating (Chaudhury and Dhadialla, 1976). It therefore seems unlikely that the mating stimulus affects the

maturation or viability of the egg.

It can be concluded that albumin is essential for follicle development and/or egg maturation in *G. p. palpalis*. However, albumin was not the only essential nutritional factor, since females fed albumin supplemented diets, expelled a significantly higher number of eggs from the uterus than control flies, and puparia from successful larvipositions were significantly smaller (Takken, in press). In frequently aborting females of *Glossina*, egg follicles 'speed up' their development, because there is no growing intra-uterine larva to inhibit the development of the next follicle in line (Saunders, 1960). Thus ovarian cycles become shorter, and the ovarian configuration is usually ahead of those in larvae-producing females of the same age. This increased rate of ovarian development was not observed in female *G. p. palpalis* fed serum substituted diets in the present investigation. In fact, the progress in ovarian configurations was similar or retarded as compared with those of control flies, indicating that follicle development is slower in the experimental flies.

The lower rate of follicle development in flies fed albumin-deficient diets compared with flies fed albumin-supplemented diets was also reflected in the rate of lipid incorporation. Both experimental diets caused marked alterations in the normal pattern of lipid changes, including a reduction in the initial rate of lipid incorporation in mated *G. p. palpalis* females, but the effect was much more pronounced in the albumin-deficient diet (Fig. 1). However, the maximum lipid level eventually reached over the 30-d period was similar for flies fed RBC + serum, RBC + saline and RBC + saline (6% BSA). Langley and Pimley (1979a) found that both the rate and the extent of accumulation of lipids by virgin or pregnant females of *G. m. morsitans* in their first and second reproductive cycle were similar for flies fed on cow blood and pig blood. However, pig blood was superior to cow blood with respect to the reproductive performance of *G. morsitans* (Mews *et al.*, 1976; Moloo and Pimley, 1978). The difference was most pronounced in the size of the puparia, and less in the fecundity. Pig-fed flies used a greater proportion of the lipid for larval nutrition than cow-fed flies, and in the latter

group some lipids apparently remained in the fly though their location was unknown (Langley and Pimley, 1979a). In the present study the fecundity was seriously impaired in both experimental diets. As the control diet and the experimental diets contained similar amounts of red blood cells, the rate of lipid incorporation (like follicle development) may, therefore, have been determined by serum constituents of which albumin was an important component. Moloo and Pimley (1978) and Takken (1980b) did not observe a beneficial effect of any serum protein except albumin on the fly's performance. The amount of albumin in the meal of *Glossina* is correlated with offspring size (Langley *et al.*, 1978; Takken, 1980b), indicating that (digested) albumin may be incorporated in the milk-gland secretion. Studies with labelled albumin in haematophagous insects have not been, to my knowledge, reported but they could answer many of the questions raised here.

It is not known which factors control lipid incorporation in tsetse flies, but there is apparently no feed-back mechanism connected with oocyte development, as flies with an impaired oocyte development can build up a lipid store equal to that of control flies (this paper). Lipids in *Glossina* are synthesized from amino acids obtained from digested blood proteins (Bursell *et al.*, 1974; McCabe and Bursell, 1975a, 1975b; Moloo, 1976). Serum albumin, in addition to its nutritive value, might provide a metabolite for the lipid-synthesizing process, which has to be produced by the albumin deficient fly, influencing the rate of lipid synthesis and incorporation in the female's fat body. In the revealance of this or some other unknown function of albumin, the existence of small amounts of complete serum albumin in the haemolymph of *G. m. morsitans* (Nogge and Gianetti, 1979) might appear to be relevant.

In flies fed normal blood, the pattern of total lipid changes is dependent on the extent and timing of larval nutrition and parturition (Langley and Pimley, 1979a; Fig. 1). The delayed ovarian development and reduced larviposition in flies fed RBC + saline (6% BSA) are related to the lower rate of synthesis and greater persistence of elevated levels of lipids than in control flies (Fig. 1). It is apparent that, although the fe-

males are capable of milk secretion, as indicated by production of viable larvae, lipids are stored at the level found in healthy pregnant females at the start of the second half of the pregnancy cycle (Langley and Pimley, 1979b).

It may be suggested that the biosynthetic activity of lipid synthesis in female *G. p. palpalis* fed on the experimental diets used in the present study is still operating, but that other essential nutritional elements, present in serum-fed flies, are absent or low in saline-fed flies. Consequently the rate, though not the extent, of lipid accumulation is reduced. This hypothesis is supported by the results of lipid fractionation and fatty acid analyses. In neutral and polar lipid fractions of flies from different feeding regimes the fatty acid compositions were quite similar (Table III) and 20-d old RBC + saline (6% BSA) fed females built up identical amounts of neutral and polar lipids as 10-d old control females (Fig. 1). Because eggs did not mature, or were non-viable (see above), the accumulated lipids remained in the fat body, but with a fatty acid composition identical to those of pregnant females. RBC + saline fed flies are either building lipids from RBC proteins, endogenous protein (amino acid) reserves carried over from their own larval stage, or both. Albumin supplementation is thus essential for egg maturation ± larval growth, but not for maximum lipid accumulation by the female.

As in *G. morsitans*, the majority of the lipids of *G. p. palpalis* consisted of neutral lipids (Cmelik *et al.*, 1969; D'Costa and Rutesasira, 1973). Palmitic, palmitoleic and oleic acid were dominant and in that aspect also differed little from the fatty acid composition of *G. morsitans* (Cmelik *et al.*, 1969; D'Costa and Rutesasira, 1973; Langley and Pimley, 1979a, 1979b). In neutral lipids of *G. p. palpalis* oleic acid relatively is greater and palmitoleic acid less than in *G. morsitans*. The only marked difference between the two species appears to be in the amount of linoleic acid in the polar lipids, which occurred at a maximum of 9.3% in 10-d and 20-d old *G. p. palpalis*, whereas D'Costa and Rutesasira (1973) found it in relative concentrations of 17.5% in *G. morsitans*.

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summary

Nutritional factors in the diet of the obligatory haematophagous tsetse fly *Glossina p. palpalis* have been investigated, as an initial step towards the development of an artificial diet for this insect. Emphasis was laid on the role of serum proteins in the reproductive physiology, particularly with respect to the development and functioning of the ovaries.

In this study flies were fed on fresh defibrinated bovine blood, in which the red cell fraction was left unchanged, but in which the serum fraction had been replaced by artificial solutions resembling serum. It is shown that *G. p. palpalis* females accept and imbibe the semi-artificial diets as readily as normal blood. Similarly diuresis and water excretion (defaecation) were not seriously impaired, especially when glucose was present in the serum substitutes (article 1). The relevance for the fly's physiology of food-acceptance, -uptake and excretion of excess water is discussed. In article 2 the effect of semi-artificial diets on *G. p. palpalis*' performance has been described. Flies were fed up to 50 or 80 days on semi-artificial diets. It was found that absence of serum albumin in the food resulted in high mortality and a seriously impaired productivity: mostly eggs and non-viable larvae were extruded. The addition of increasing levels of serum albumin improved the fecundity until it was similar to that of flies fed on a control diet. The serum albumin concentration in the diet appeared to be positively correlated with the size of newly produced larvae. It was suggested that albumin was an essential nutritional factor in the diet of *G. p. palpalis*.

To investigate the physiological effects of serum albumin on the reproductive performance, flies were fed on semi-artificial diets, which were prepared differently from those used in the previous experiments because the new diets were reconstituted from a mixture of bovine red cells and serum-substitutes that previously had been lyophilized. The composition of the

lyophilized diets, however, was the same as used in the previous experiments. On a serum albumin deficient diet, egg maturation and ovulation were seriously impaired and egg follicles were observed to be retained and lysed in the ovaries (article 3). Flies fed on serum albumin containing diets ovulated, but the fecundity was low and puparia were smaller than those obtained from control flies. It is discussed how dietary substances might affect egg maturation, ovulation and egg hatching.

Article 4 describes the results of a study on the rate of ovarian development and lipid incorporation and the fatty acid composition of flies. Fed on semi-artificial diets over a 30-day period. Ovulation did not take place in flies fed serum albumin deficient diets, while flies fed serum albumin containing diets ovulated and produced larvae though at a lower rate than control flies. Compared with control flies, lipid incorporation was reduced in experimental flies, but more in the albumin deficient group than in the albumin containing group. Dietary composition had no marked influence on the relative fatty acid composition in any one group.

In general, it is concluded that serum albumin is a highly essential nutritive element for cow-blood fed *G. p. palpalis*, without which flies fail to reproduce and have a high mortality.

samenvatting

Als een eerste stap in de ontwikkeling van een kunstmatig dieet voor de obligaat hematofage tsetse vlieg *Glossina p. palpalis* werden voedingsfaktoren in het dieet van dit insect onderzocht. De nadruk werd gelegd op de rol van serumproteïnen in de voortplantingsfysiologie, vooral met betrekking tot de ontwikkeling en het funktioneren van de ovaria. In dit onderzoek werden vliegen gevoed met vers gedefibrineerd runderbloed, waarin de rode celfraktie onveranderd was gelaten, maar waarin de serumfraktie was vervangen door kunstmatige oplossingen die op serum leken. Aangetoond werd, dat *G. p. palpalis* vrouwtjes de gedeeltelijk kunstmatige diëten even goed accepteerden en opnamen als normaal bloed. Evenzo werden de diurese en de wateruitscheiding (defecatie) niet in ernstige mate geremd, speciaal wanneer glucose aanwezig was in de serumsubstituten (artikel 1). De relevantie voor fysiologische processen zoals de voedselacceptatie en -opname en de uitscheiding van overtollig water door de vlieg werd besproken.

In artikel 2 werd het effect van kunstmatige diëten op de overlevingsduur en voortplanting van *G. p. palpalis* beschreven. Vliegen werden 50 of 80 dagen met gedeeltelijk kunstmatige diëten gevoed. De afwezigheid van serumalbumine in het voedsel resulteerde in een hoge mortaliteit en remde de produktiviteit aanzienlijk; vooral in het ei- en het larvestadium was er sprake van een hoge mortaliteit. De toevoeging van toenemende hoeveelheden serumalbumine deed de vruchtbaarheid toenemen, tot deze gelijk was aan die van de vliegen die gevoed werden met een controle dieet. De concentratie van serumalbumine in het dieet bleek positief gecorreleerd te zijn met de grootte van de nieuw geproduceerde larven. Gesuggereerd werd, dat albumine een essentieel nutriënt was voor *G. p. palpalis*.

Teneinde de fysiologische effekten van serumalbumine op de voortplanting te onderzoeken, werden vliegen gevoed met gedeeltelijk kunstmatige diëten, die op een andere wijze werden bereid

dan die welke gebruikt werden in de voorgaande experimenten. De nieuwe diëten werden samengesteld uit een mengsel van rode bloedcellen afkomstig van runderen en serumsubstituten, die in een eerder stadium gevriesdroogd waren. De samenstelling van deze diëten was echter gelijk aan die welke gebruikt werd in de voorafgaande experimenten. Bij een serumalbumine deficiënt dieet werden de eirijping en de ovulatie ernstig geremd en werd waargenomen, dat eifollikels werden vastgehouden en werden opgelost in de ovaria (artikel 3). Vliegen die gevoed werden met serumalbumine bevattende diëten ovuleerden, maar de vruchtbaarheid was laag en de puparia waren kleiner dan die welke verkregen werden uit controle vliegen. Besproken werd, hoe dieetcomponenten mogelijk de eirijping, de ovulatie en het uitkomen van de eieren kunnen beïnvloeden.

Artikel 4 beschrijft de resultaten van een onderzoek naar de ontwikkelingssnelheid van de ovaria, de lipide incorporatie en de vetzuursamenstelling van vliegen die gevoed werden met gedeeltelijk kunstmatige diëten gedurende een periode van 30 dagen. In vliegen die gevoed werden met een serumalbumine deficiënt dieet vond geen ovulatie plaats, terwijl vliegen die gevoed werden met serumalbumine bevattende diëten ovuleerden en larven produceerden, hoewel met een lagere snelheid dan controle vliegen. Vergeleken met controle vliegen werd in experimentele vliegen de lipide incorporatie gereduceerd, maar dit gebeurde sterker in de albumine deficiënte dan in de albumine bevattende groep. De dieetsamenstelling had in geen van de groepen een merkbare invloed op de relatieve vetzuursamenstelling.

In het algemeen werd geconcludeerd, dat serumalbumine een zeer essentieel voedingsbestanddeel is voor met runderbloed gevoede *G. p. palpalis*. Zonder serumalbumine kunnen de vliegen zich niet voortplanten en is de mortaliteit hoog.

CURRICULUM VITAE

Willem Takken werd geboren op 5 januari 1951 te Lochem. Na het behalen van het eindexamen Gymnasium bèta in 1969 aan het Baudartius College te Zutphen begon hij zijn studie aan de Landbouwhogeschool te Wageningen. Hij legde het kandidaatsexamen Planteziektenkunde in juni 1973 af, waarna hij in september 1976 het doctoraal examen Planteziektenkunde (met de vakken entomologie/parasitologie, biochemie en toxicologie) behaalde. In de periode september 1976 tot mei 1977 was hij in dienst van de Landbouwhogeschool werkzaam voor de vakgroep Toxicologie. Hij was in dit verband betrokken bij een studie naar de neveneffekten van de chemische bestrijding van tsetse vliegen in West Afrika. Van juni 1977 tot juni 1980 was hij in dienst van de FAO werkzaam op het laboratorium van de Joint FAO/IAEA Division of Isotope and Radiation Applications of Atomic Energy for Food and Agricultural Development, Postbus 200, A-1400 Wenen, Oostenrijk. Hij werkte mee aan de ontwikkeling van een massa kweekmethode voor tsetse vliegen en aan een studie naar de effekten van bestraling op de fysiologie van het gedrag van de tsetse vlieg. Hij is sinds juli 1980, wederom in dienst van de FAO, werkzaam in Mozambique.*

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