Pteridines in flies of the genus "Glossina" (Diptera)

Autor(en): **Harmsen, Rudolf**

Objekttyp: **Article**

Zeitschrift: **Acta Tropica**

Band (Jahr): **27 (1970)**

Heft 2

PDF erstellt am: **13.09.2024**

Persistenter Link: https://doi.org/10.5169/seals-311637

Zoology Department, University College, Nairobi, Kenya

Pteridines in Flies of the Genus *Glossina* (Diptera)

RUDOLF HARMSEN *

It is as yet not known which factors of the micro environment of the sleeping sickness causing trypanosomes stimulate the morphogenesis and growth of these flagellates inside the tsetse fly. The marked effect of biopterin and biopterin derivatives on the growth of another flagellate: the mosquito parasitic *Crithidia fasciculata* (ZIEGLER & NATHAN, 1961) suggest a possible pterine growth and morphogenetic effect on *Trypanosoma* spp.

The closest relatives of the tsetse flies that have been investigated for pteridine content are flies of the genera *Drosophila* (ZIEGLER & HADORN, 1958; HUBBY & THROCKMORTON, 1960; ZIEGLER, 1961), *Calliphora* (ZIEGLER, 1961), *Phormia, Musca, Pollenia, Ceratitis* (ZIEGLER, 1960; 1963) and *Aedes* and *Culex* (BHALLA, 1968) (see Table 1). All these flies seem to have the biopterin group of pterines well represented, although in relatively low concentrations except in the eyes. The biosynthesis of tetrahydrobiopterin occurs in the eyes and probably also in other parts of the body. Biotransformation and part oxidation of the hydrogenated pterines in the eyes results in an accumulation of biopterin, sepiapterin and in some cases of the three drosopterins (cf. ZIEGLER & HARMSEN, 1969), most of which possess a *Crithidia* effect. A more drastic biodegradation of tetrahydrobiopterin and/or folic acid results in the presene of a number of simple pterines: isoxanthopterin, xanthopterin, pterin (2-amino-4-hydroxy pteridine), and pterin-6-carbonic acid. None of these substances have a growth stimulating effect on *Crithidia,* neither has riboflavine, which has also been isolated from Dipteria. The distribution of pterines in *Diptera* is summarized in Table 1.

The purpose of this investigation was to determine the presence and abundance of pteridines in a number of species of *Glossina* (the tsetse flies).

Materials and Methods

Only adult tsetse flies of known age were used for the experimental work. Most of the work was done with *Glossina pallidipes,* but some other species *(longipennis, brevipalpis, morsitans, palpalis, austeni* and *fusca)* were cursorily examined for possible differences. The *G. pallidipes* were collected at Kiboko and Lambwe, Kenya by personnel of the Kenya Department of Agriculture, Tsetse Division. The other species were collected at various locations in Kenya and Uganda. All flies were collected as puparia, and allowed to emerge in the laboratory. For extraction purposes the flies were either freshly killed and used immediately, or stored at $-10°C$ until use.

Standard samples of riboflavine and all chromatographic reagents were obtained from B.D.H. (England); kynurenine, xanthopterin and leucopterin from Light + Co (1961); isoxanthopterin, biopterin, pterin-6-carbonic acid, pterin-7-carbonic acid and pterin samples were donated by Professor M. VISCONTINI (1961); the drosopterins were isolated from wild-type *Drosophila melanogaster;*

* Present address: Biology Dept., Queen's University, Kingston, Canada.

sepiapterin and isosepiapterin from *D. melanogaster* mutant *sepia;* and erythropterin from the wings of *Catopsilia arganthe.*

The extraction and separation techniques employed were basically the same as those described by HARMSEN (1963, 1966). Whole flies, whole heads, isolated fat bodies, or headless flies were homogenized in a mixture of chloroform and an aqueous solvent in air tight glass homogenizers in the dark. In the case of eyes, 40 eyes were dissected out of the flies, and homogenized with 0.5 ml 1% ammonia and 0.5 ml chloroform. The homogenate, after centrifugation, carries all pteridines in the aqueous supernatant. Small quantities in multiples of 2 μl volumes of this supernatant were used for two-dimensional descending chromatographic separation on Whatman No. 1 paper. All separations were performed in the dark. For the first dimension the following solvents were used: n-propanol/water (7:3); n-propanol/1% ammonia (7:3); methanol/water (7:3); methanol/1% ammonia (7:3); n-butanol/water/glacial acetic (4:1:1); water saturated collidine. For the second dimension the following solvents were used: 0.1M sodium citrate; 5% formic acid; 20% potassium chloride; 3% ammonium chloride. Best results were obtained with n-propanol/1% ammonia (or water where the presence of erythropterin was suspected) for the first dimension, and 0.1M sodium citrate for the second dimension.

Chromatograms were observed in UV-light of both 365 mμ and 265 mμ, pterines fluoresce in the former wavelength, purines absorb light of the shorter wavelength. Substances can be identified by their Rf values in various solvents, by their colour of fluorescence at various pH levels and in some cases by their colour when viewed in white light. Also the oxidative effect of UV light and identification of the breakdown products can be used for the identification of certain pterines.

The use of UV-spectrophotometry for the characterization and/or identification of pterines has been limited to isoxanthopterin and the most abundant red-red fluorescing eye pterine: PR-1. Only the elution of 16 chromatograms, subsequent concentration and second chromatography gave an adequate amount of concentrated material for spectrophotometric analysis.

Results

The fatbody and headless whole fly extracts contain only very small quantities of biopterin and isoxanthopterin as well as traces of other unidentified fluorescing substances. On chromatograms of extracts of eyes of *Glossina pallidipes* a total of 16 consistently occurring fluorescing spots can be recognized (see Fig. 1). Six blue or purple fluorescing spots, three gree ones, and seven red, orange or yellow ones. Several other fluorescing spots were encountered irregularly, and usually at very low intensity.

An analysis of the fluorescing spots revealed the following information.

Pb-6: This spot corresponds completely with the one of synthetic biopterin. Its fluorescence colour and photolability are consistent with those of biopterin.

Pb-2: Fluorescence and Rf values correspond completely with those of isoxanthopterin.

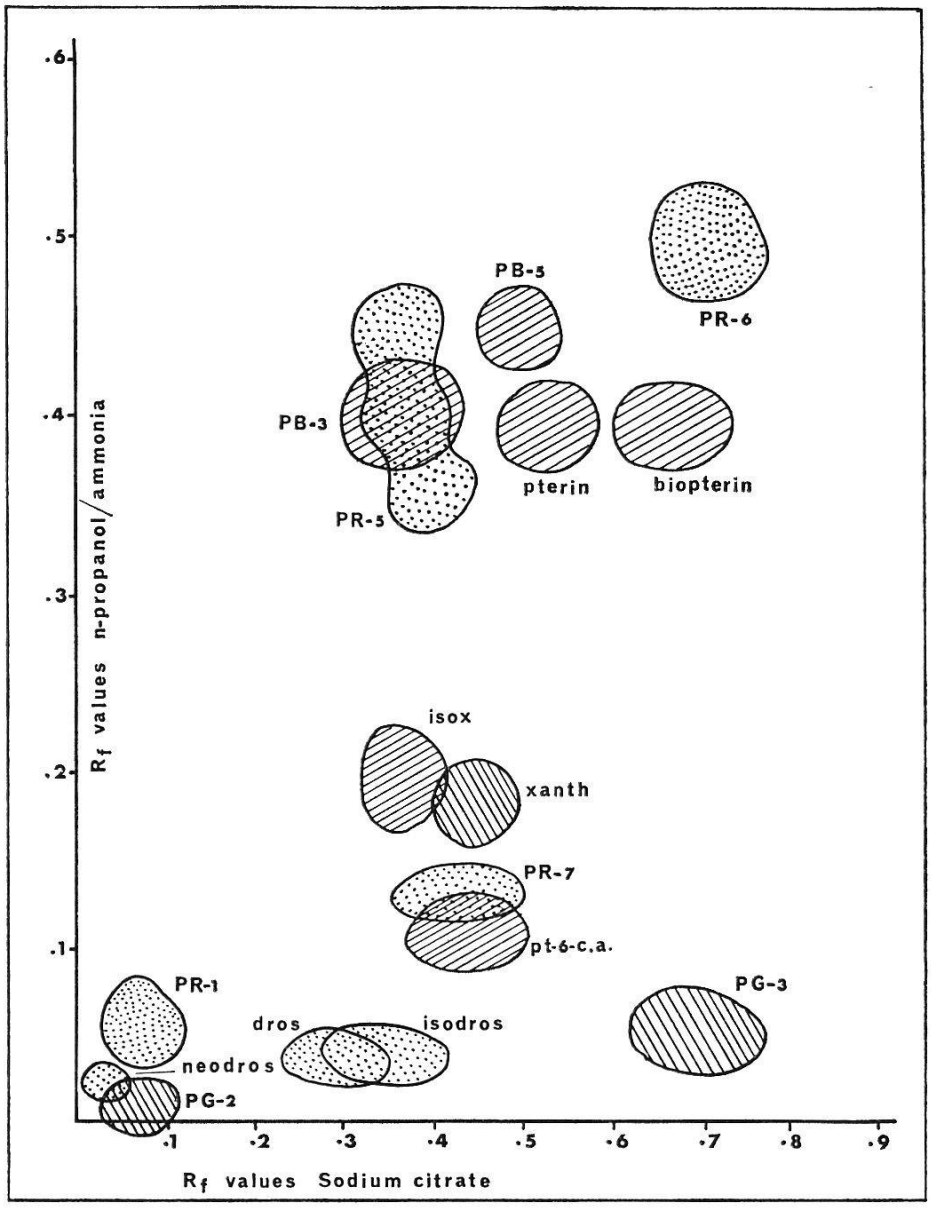

Fig. 1. Two dimensional chromatograph of eye extract of *Glossina pallidipes.*

Pb-4: Fluorescence and Rf values correspond with those of pterin.
Pb-1: This spot corresponds in all aspects with pterin-6-carbonic acid.
Pb-3: This spot was also identified as of pterin-6-carbonic acid. The spot only appeared if the chromatogram was viewed under U.V. light after the first dimension run, and, therefore, represents a breakdown product of biopterin.

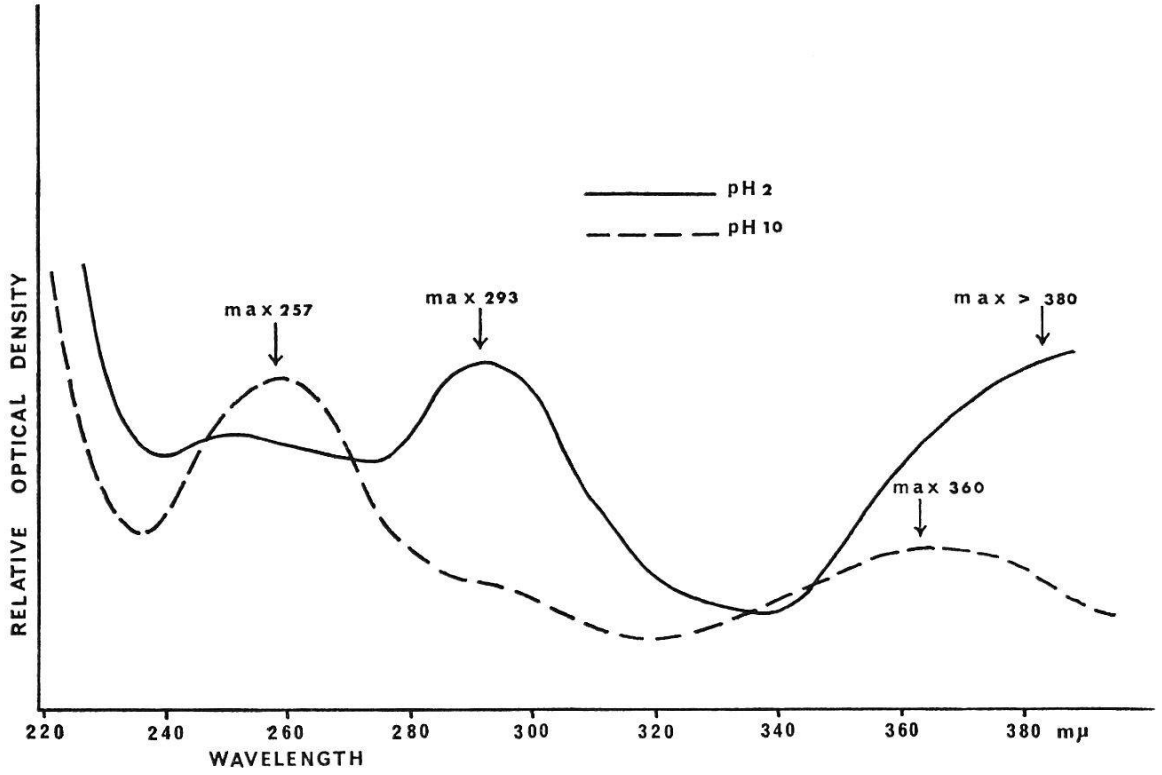

Fig. 2. U.V.-Absorption spectrum of pterine-like substance Pr-1 from the eyes of *Glossina pallidipes*.

Pb-5: Identification of this spot is tentative, but it is expected to be also of pterin and is probably a chromatographic degradation product of Pr-6 (isosepiapterin).

Pg-1: This somewhat irregularly occurring spot is identified as representing xanthopterin.

Pg-2 and Pg-3: These two green fluorescing substances have not been identified. Pg-2 may not be a pterine.

Pr-2, Pr-3 and Pr-4: These three substances have been identified respectively as drosopterin, isodrosopterin and neodrosopterin. This identification is based on a comparison of the chromatographic behaviour and fluorescence of these substances and the known substances from *Drosophila* eye extracts. The individual identification is based on the published Rf values of the three substances (ZIEGLER & NATHAN, 1961; VISCONTINI, 1963).

Pr-5: This yellow to orange fluorescing spot probably represents riboflavine and/or sepiapterin. No definite identification was possible.

Pr-6: This yellow fluorescing substance may be isosepiapterin, but a definite identification was not possible.

Pr-7: A stable, bright orange fluorescing substance. No identification was possible; it does not correspond to any known substance.

Pr-1: This is the most abundant orange fluorescing substance in tsetse fly eyes. It is not identifyable with any known substance, but may

correspond to an unidentified substance isolated from *Plodia interpunctella* (Almeida, 1958).

In summary, the pterines occurring in *Glossina* are included in Table 1. Of the four unknown, new substances of possible pterine nature, Pr-1 alone was encountered in sufficient concentration to allow spectrophotometric analysis. UV-absorption spectra of this substance were made at both pH_2 and pH_{10} (see Fig. 2).

The species of *Glossina* other than *G. pallidipes* that were examined differed in no significant way from *pallidipes*. The only noticible difference being that the larger species (*brevipalpis, longipennis,* and *fusca*) appeared to lack all traces of the drosopterins. In these species, however, Pr-1 is strongly present.

TABLE 1

Pteridines in Diptera, emphasizing those substances of possible growth and morphogenetic effect in Trypanosomes

	Pterin	Xanthopterin	Isoxanthopterin	Leucopterin	Pterin-6-c.a.	Tetrahydrobiopterin	Biopterin	Sepiapterin	Drosopterines	Isosepiapterin	Riboflavine	C-7-subst. pterines	Pr-1
Aedes aegypti	(+)						+	+			+		
Aedes mascariensis	(+)						+	+			+		
Calliphora erythrocephala			+		(+)	+	?	+					
Ceratitis capitata	(+)		+		(+)	+	+	+					
Culex pipiens	(+)						+	+			+		
Drosophila melanogaster	(+)	+	+	–	(+)	+	+	+	+	(+)	+	–	
Drosophila (other spp.)	(+)	+	+			?	+	+/–					
Musca domestica	(+)		+		(+)	+	+						
Phormia regina	(+)		+		(+)	+	+						
Pollenia viridis	(+)		+		(+)	+	+						
Glossina pallidipes	(+)	+	+	–	(+)	?	+	+	+	?	?	–	+
						substances with *Crithidia* effect							

\+ positively identified; recognized as naturally occurring substance
(+) positively identified; suspected of being artificial degradation product
? presence suspected
– searched for with negative result.

Discussion and Conclusions

The presence of a number of C-6 substituted pterines, concentrated in the eyes of *Glossina pallidipes* shows that the general situation in tsetse flies is similar to that in other Diptera. The biopterin may well be, in part at least, a degradation product of tetrahydrobiopterin, particularly in the case of biopterin in the body. The extraction technique employed would probably result in the oxidation of any tetrahydrobiopterin present. The presence of small quantities of all three drosopterins, and probably also of sepiapterin and isosepiapterin in the eyes shows that the typical Dipteran eye dehydrogenation sequence and pigment deposition is present in *Glossina*. Pr-1 probably belongs to this group of pterines, and has replaced the drosopterins as the main red eye pigment.

Pterin and isoxanthopterin are probably the only naturally occurring simple pterines. Pterine-6-carbonic acid is probably a dregradation product of biopterin. The position of xanthopterin is uncertain. This substance is usually associated with C-7 substituted pterines, a group which is conspicuously absent in the tsetse fly. However, xanthopterin has also been reported from *Drosophila* (ZIEGLER & HADORN, 1958). In *Diptera,* it is probably the oxidative breakdown product of the naturally occurring 7,8-dihydroxanthopterin (HARMSEN, 1969).

It is hoped that future work will result in the identification and characterization of the apparently new substances (Pr-1, Pr-7, Pg-3) of possible pterine nature. However, without these identifications it can already be said that the tsetse flies have a measurable content of *Crithidia* active pterines in the entire body, and a concentration of these substances in the eyes. The concentration of these pterines in the tsetse fly is at least an order of magnitude higher than it is in mammalian blood. The availability of these substances to trypanosomes of the *brucei* species complex inhabiting the fly is, of course, not established, but it is likely that at least in the salivary gland this is the case. It is suggested, that hydrogenated C-6 substituted pterines may very well play a role in growth and morphogenesis of *brucei* trypanosomes in the tsetse fly.

Acknowledgements

I wish to record my gratitude to the Director, Kenya Veterinary Research Laboratory, for supplying the *G. pallidipes* used in this work and to the Rockefeller Foundation for making a grant for research to the Science Faculty, University College, Nairobi through which this research was supported. The excellent technical assistance of Miss I. Jabbal was of much value to the work.

References

BHALLA, S. C. (1968). Genetic aspects of pteridines in mosquitoes. – Genetics, Princeton *58,* 249–258

HARMSEN, R. (1963). The storage and excretion of pteridines in *Pieris brassicae* L. and some other insects. – Thesis, Cambridge

HARMSEN, R. (1966). Identification of fluorescing and U.V. absorbing substances in *Pieris brassicae* L. – J. Insect Physiol. *12,* 23–30

HARMSEN, R. (1969). The effect of atmospheric oxygen pressure on the biosynthesis of simple pterines in pierid butterflies. – J. Insect Physiol. *15,* 2239–2244

HUBBY, J. L. & THROCKMORTON, L. H. (1960). Evolution and pteridine metabolism in the genus *Drosophila.* – Proc. nat. Acad. Sci. U.S.A. *46,* 65–78

VISCONTINI, M. (1963). Ptérines et génétique. – Il Farmaco *18,* 47–64

ZIEGLER, I. (1960). Tetrahydrobiopterin-Derivat als lichtempfindliche Verbindung bei Amphibien und Insekten. – Z. Naturf. *15b,* 460–465

ZIEGLER, I. (1961). Zur genphysiologischen Analyse der Pterine in Insektenaugen (*Drosophila melanogaster* und *Calliphora erythrocephala*). – Z. Vererbungsl. *92,* 239–245

ZIEGLER, I. (1963). Reinigung des Tetrahydrobiopterins aus Insektenaugen. – Biochim. biophys. Acta *78,* 219–220

ZIEGLER, I. & HADORN, E. (1958). Manifestation rezessiver Augenfarbgene im Pterin-Inventar heterozygoter Genotypen von *Drosophila melanogaster.* – Z. Vererbungsl. *89,* 235–245

ZIEGLER, I. & HARMSEN, R. (1969). The biology of pteridines in insects. In: Advances in Insect Physiol. (Eds. J. W. L. Beament, J. E. Treherne & V. B. Wigglesworth), pp. 139–203. – London/New York: Academic Press

ZIEGLER, I. & NATHAN, H. A. (1961). Wuchsstoffaktivität der Augenpterine von *Drosophila melanogaster* bei *Crithidia fasciculata.* – Z. Naturf. *16b,* 260–262

Zusammenfassung

Die Bedingungen, welche für die morphogenetische Entwicklung der Trypanosomen notwendig sind, sind unbekannt. Pteridine zeigen eine essentielle Wuchswirkung auf Trypanosomiden der Gattung *Crithidia.* Die Trypanosomen der Säugetiere mögen wohl für die Vollendung ihres Lebenszyklus Substanzen von der Art der Pteridine im Insektenwirt brauchen. Viele Dipterenarten enthalten hohe Konzentrationen von Pteridinen.

Fliegen der Gattung *Glossina* wurden untersucht. Sieben bekannte Pteridine wurden definitiv bestimmt, und die Anwesenheit vier weiterer wird vermutet. Zwei neue Pteridine wurden isoliert und kurz beschrieben, aber sie wurden noch nicht chemisch analysiert.

Besonders Biopterin und der vielleicht damit verwandte, neue Pr-1-Stoff sollten auf ihre mögliche Wuchswirkung und auf ihre Bedeutung als morphogenetische Faktoren für Trypanosomen der Säuger untersucht werden.

Résumé

Les stimulus nécessaires pour le développement morphologique des trypanosomes dans la mouche tsé-tsé sont inconnus. Les ptéridines ont un effet important sur la croissance des trypanosomides du genre *Crithidia.* Il est possible que les trypanosomes des mammifères requièrent des substances telles que les

ptéridines de leur hôte pour compléter leur cycle de développement. Plusieurs espèces de diptères contiennent de hautes concentrations de ptéridines.

Des mouches du genre *Glossina* ont été étudiées. Sept ptéridines connues ont été identifiées et la présence de quatre autres est soupçonnée. Deux nouvelles ptéridines ont été isolées et sont brièvement décrites mais n'ont pas été analysées chimiquement.

La bioptérine et la nouvelle substance Pr-1 qui lui est peut-être apparentée devraient être étudiées comme facteurs possibles de croissance et de morphogénèse pour les trypanosomes des mammifères.