Miscellaneum : The stimulation of emergence from Glossina pupae with gamma rays

Autor(en): **Curtis, C.F.**

Objekttyp: **Article**

Zeitschrift: **Acta Tropica**

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

Heft 1

PDF erstellt am: **17.05.2024**

Persistenter Link: https://doi.org/10.5169/seals-311634

Miscellaneum

The Stimulation of Emergence from Glossina Pupae with Gamma Rays

By C. F. Curtis

Tsetse Research Laboratory, University of Bristol, School of Veterinary Science, Langford, Bristol, England

In the course of studies on radiation sterilisation of pupae of *Glossina austeni* and *G. morsitans,* which were close to the age of spontaneous emergence, it was unexpectedly found that gamma radiation stimulated emergence from many of the pupae within two minutes of the treatment. Some investigations of this phenomenon was made in the hope of obtaining an indication of its mechanism and with a view to its possible use as an experimental technique.

Material and methods

The pupae used were deposited on a known date by members of the colonies of *G. austeni* and *G. morsitans* maintained in this laboratory. The pupae were kept in pots of sand at about 24°C and 80% r.h. except during the irradiation.

Irradiation was with ^{60}Co gamma rays. In most of the experiments the radiation was provided by a high intensity machine. Samples were lowered to the centre of the source by a lift. Lowering and raising the lift occupied 15 seconds and during these operations the dose rate received by the pupae varied from zero to a maximum of 14,000 rads/minute. In most experiments, in order to give the minimum possible dose, raising of the lift was started as soon as the pupae reached the centre of the source and during the lowering and raising a total of 700 rads were received by the pupae, as shown by measurement of the optical density, at 305 mμ, of a 0.001 M ferrous sulphate solution irradiated at the same time. In certain experiments the pupae were allowed to remain at the region of maximum dose rate for a measured time interval.

In one experiment a radiotherapy source with a dose rate of 68 rads/minute was used.

Results

Pupae of *G. austeni* and *G. morsitans* which had been deposited in the same 24 hour period were divided into two groups, A and B, and the number of flies of each sex emerging from each group was recorded, generally in each 12 hour interval (at 11.00 and 23.00 hours). The results are shown in Table 1. Using the high intensity machine, group B of *G. morsitans* was irradiated 29.5 days after deposition, i.e. when about 17% of individuals had emerged spontaneously, and group B of *G. austeni* was irradiated at 31.5 days, i.e. when about 10% had emerged spontaneously. As shown in Table 1, a considerable proportion of individuals were induced to emerge at the time of irradiation, and comparison of the pattern of emergence subsequent to irradiation in group B, with that in the untreated group A, indicates that in both species the irradiation stimulated those individuals of each sex to emerge that would have done so spontaneously within 1–4 days. The same was also found to apply in other experiments with *G. austeni*

Table 1. Times of emergence of male and female *G. morsitans* and *G. austeni* pupae. Groups B were irradiated at the times indicated; Groups A were unirradiated controls

Time of observation (days from deposition)	*G. austeni*				*G. morsitans*			
	Group A		Group B		Group A		Group B	
	♂	♀	♂	♀	♂	♀	♂	♀
26.5	–	–	–	–	–	–	–	–
27.5	–	–	–	–	–	1	–	1
28.5	–	–	–	–	–	4	–	2
29.5	–	–	–	1	–	2	–	2
							Irradiation:	
							2	7
30.0	–	–	–	–	–	3	–	–
30.5	–	2	1	1	2	5	–	–
31.0	–	–	–	–	–	–	–	–
31.5	–	5	–	6	5	–	2	–
			Irradiation:-					
			5	38				
32.0	–	6	–	–	5	–	4	1
32.5	1	8	–	–	6	–	7	–
33.0	1	7	–	–	2	–	1	–
33.5	3	6	8	–	1	–	4	–
34.0	3	1	4	–	–	–	–	–
34.5	3	5	5	–	–	–	–	–
35.0	8	1	8	–	–	–	–	–
35.5	6	3	5	–	–	–	–	–
36.0	6	–	1	–	–	–	–	–
36.5	4	–	–	–	–	–	–	–
37.0	1	–	–	–	–	–	–	–
39.0	–	–	–	–	–	–	–	–
Unhatched	6		4		0		4	
Total pupae	86		87		36		37	

when the irradiation was given after about 1%, and about 30%, of individuals had emerged spontaneously – in the latter case the radiation stimulated emergence from all the remaining viable pupae.

In the experiments so far described the radiation was given in successive doses of 700 rads with a pause of a few minutes between each. With *G. morsitans* no individuals emerged until after the second dose and there was no further response to two more doses. With *G. austeni* most emergence occurred after one dose, a few more responded after two, three or four doses, but a fifth dose gave no further response. A single dose of 2800 rads was equally effective in stimulating emergence and additional doses gave no further effect. In *G. morsitans* the ptili-

Table 2. Number of flies that had fed at least once in their life time over the total of flies tested, after offering blood meals on four successive occasions

Time after emergence (hr)	*G. austeni*		*G. morsitans*	
	Spontaneous emergence	Radiation stimulated emergence	Spontaneous emergence	Radiation stimulated emergence
2½	0/20	3/30	5/10	1/9
5½	2/14	5/22	4/8	4/7
24	6/10	11/15	5/5	4/5
48	5/5	6/7	2/2	2/3

num broke through the puparium within a few seconds of the end of the radiation treatment, but in *G. austeni* there was a delay of ½–2 minutes.

To test the effect of a much lower dose rate, some *G. austeni* pupae were exposed to a radiotherapy source. Most had emerged by the time 400 rads had been given, but a few did not respond until the total dose received was 1400 rads. These results are consistent with those obtained with the high intensity machine and it therefore appears that a given number of rads have the same effect on emergence over a wide range of dose rates.

It seemed possible that the stimulation of emergence, that was found, was an effect of temperature changes associated with the process of irradiation. However, no emergence occurred during transport of the pupae to or from the ^{60}Co machines or on returning them to the heated insectary – all stimulated emergence occurred within two minutes of the radiation treatment. During one of the experiments in which stimulation of emergence occurred, the interior of the high intensity machine was cooled with circulating methanol to about 20°C. A thermocouple (buried in a volume of sand approximately the same as the volume of the pupae that were treated) was inserted into the source. It showed no detectable change from room temperature in a half minute's exposure, which was longer than that used for the pupal treatments. Thus the stimulation of emergence must have been due to the radiation itself.

After radiation stimulated emergence the flies expanded their wings normally. They were offered blood meals by strapping cages of the flies to a rabbit's ear for 15 minute periods on four occasions in the first two days after emergence. The numbers that had taken at least one feed in their life time are shown in Table 2 with the numbers of flies present on each occasion (this number declined because some flies were fixed on each occasion for histological examination). For comparison, similar observations were made on flies that had emerged spontaneously within a one hour period. The readiness to feed appears to develop at about the same rate from the time of emergence in the radiation stimulated and the control groups.

It is well known to tsetse workers that emergence from mature pupae can be stimulated by pricking the anterior end of the puparium with a needle (A. M. JORDAN, personal communication). In a batch of *G. morsitans* pupae of which 20% had emerged spontaneously, 7 out of 10 unhatched pupae were induced to emerge by pricking. This is a higher proportion than that which responded to irradiation at about the same age (Table 1). In *G. austeni* some pupae that did not respond when irradiated were immediately afterwards induced to emerge by pricking, and it therefore appears that the latter treatment can induce emergence from pupae at an earlier stage of development than is the case with radiation.

Discussion

The phenomenon of radiation stimulated emergence was encountered by DEAN, WILSON & WORTHAM (1968) who irradiated *G. morsitans* pupae of mixed ages collected from the wild and recorded that "dosages above 4000 rads increased the rate of eclosion during and immediately after irradiation, while it was generally reduced during the next day". Nothing else appears to be known about the stimuli causing emergence from *Glossina* pupae apart from observations on circadian rhythms by BURSELL (1959, 1960) and DEAN *et al.* (1968). There is little indication of the mechanism by which gamma radiation stimulates emergence from the experiments described, but the fact that the effect depends only on total dose received and not on dose rate, or on whether the dose was fractionated, suggests that the effect is due to production or destruction by radiation of a chemical intermediate which promotes or inhibits emergence from the pupae, rather than to direct stimulation of the nervous system. If this is a correct interpretation, the response is remarkably rapid, especially in *G. morsitans*.

Radiation stimulation of emergence might prove useful if pupae of assorted ages were available and batches of freshly emerged flies were required for experimental purposes at particular times. It would presumably be possible to re-irradiate at 2–3 day intervals to stimulate emergence from those pupae that had in the meanwhile reached the appropriate age. *G. morsitans* pupae aged more than 16 days show no mortality as a result of an 8000 rad treatment (DEAN *et al.*, 1968), so that six or more applications of the stimulating dose could be given without killing any of these more mature pupae.

Tsetse can only be infected with the *brucei* group of trypanosomes if given an infected feed very early in adult life (WIJERS, 1958; WARD, 1968). The fact that very young flies can be infected apparently results from the incomplete development of the peritrophic membrane at this time (WILLETT, 1966). Even when an infected feed is given on the first day of adult life the proportion of flies that become infected is inconveniently low for experimental purposes. It seems possible that by stimulating emergence at an usually early age, by irradiation or pricking of the puparium, newly emerged flies might have exceptionally immature peritrophic membranes and hence be more readily infected than usual. It may be, however, that the stage of development of the membrane seen at the time of natural emergence has already been reached in the pharate adult, and that the act of emergence supplies the stimulus for maturation of the membrane to begin. If this is the case, artificial induction of emergence would not be expected to increase the trypanosome infection rate.

Acknowledgements

The high intensity ^{60}Co machine was made available by the Meat Research Institute, Langford. I am very grateful to Mr. S. Dant for operating the machine. The radiotherapy source was made available by Bristol General Hospital.

I am grateful to Drs. T. A. M. Nash and A. M. Jordan for criticizing the manuscript.

Financial support was provided by the British Ministry of Overseas Development.

References

BURSELL, E. (1959). The water balance of tsetse flies. – Trans. roy. ent. Soc. Lond. *111*, 205–235

BURSELL, E. (1960). The effect of temperature on the consumption of fat during pupal development in *Glossina*. – Bull. ent. Res. *51*, 583–598

DEAN, G. J.; WILSON, F. & WORTHAM, S. (1968). Some factors affecting eclosion of *Glossina morsitans* from pupae. – Bull. ent. Res. *58*, 367–377

WARD, R. A. (1968). The susceptibility of *Glossina austeni* to infection with *Trypanosoma brucei*. – Trans. roy. Soc. trop. Med. Hyg. *62*, 672–678

WIJERS, D. J. B. (1968). Factors that may influence the infection rate of *Glossina palpalis* with *Trypanosoma gambiense*. I. The age of the fly at the time of the infected feed. – Ann. trop. Med. Parasit. *52*, 385–390

WILLETT, K. C. (1966). Development of the peritrophic membrane in *Glossina* (Tsetse flies) and its relation to infection with trypanosomes. – Exper. Parasit. *18*, 290–295