

was proven by inhibitory effect of  $\alpha$ -methylmannose. In the presence of 100  $\mu$ M of  $\alpha$ -methylmannose, nonspecific binding was found to be about 2% of the total binding. The values obtained for the total binding were therefore routinely corrected by this amount.

Figure 2 shows that at a concentration of 600 ng/assay mixture, binding of lentin to blastula chromatin was directly proportional to chromatin concentration up to about 1  $A_{260}$  unit, and that, at higher concentration of chromatin, it tended to level off.

Data presented in the inset of figure 3 show that the binding of lentin to chromatin is saturable. Maximal binding of about 150 ng of lentin is achieved at approx. 600 ng of lentin/1  $A_{260}$  unit of chromatin. Scatchard plot analysis of the binding reaction suggested a single type of binding site.

In view of a strong affinity of lectins towards the sugar residues, one would expect the presence of bound carbohydrates in chromatin. These compounds were indeed detected in chromatin by a variety of techniques<sup>9-12</sup>. We also found that nonhistone proteins (isolated from chromatin prepared by the procedure used here) exhibit a positive reaction with periodic acid-Schiff (PAS) reagent. The glycoproteins were found to reside among the high (> 67,000) and the low (< 18,000) mol. wt proteins<sup>13</sup>. The high mol. wt nonhistone proteins were identified as concanavalin A receptors by Rizzo and Bustin<sup>2</sup>. While these authors pro-

pose the use of lectins as structural probes for the study of organization of a restricted class of components in chromatin, we suggest that the lectin-binding property of chromatin could be exploited to provide new insights into the role of nonhistone glycoproteins in the regulation of gene activity.

- 1 N. Sharon and H. Lis, *Science* 177, 949 (1972).
- 2 W. Rizzo and M. Bustin, *J. biol. Chem.* 252, 7062 (1977).
- 3 R. Jackson, *Biochemistry* 15, 5652 (1976).
- 4 D. E. Comings and D. C. Harris, *Exp. Cell Res.* 96, 96 (1975).
- 5 S. Penman, *J. molec. Biol.* 17, 117 (1966).
- 6 E. Holtzman, J. Smith and S. Penman, *J. molec. Biol.* 17, 131 (1966).
- 7 F. H. Wilt and E. Ekenberg, *Biochem. biophys. Res. Commun.* 44, 831 (1971).
- 8 C. L. Fitzmaurice and F. R. Baker, *Biochem. biophys. Res. Commun.* 55, 328 (1973).
- 9 Lj. Ševaljević and K. Krtolica, *Int. J. Biochem.* 4, 345 (1973).
- 10 D. Tuan, S. Smith, J. Folkman and E. Merler, *Biochemistry* 12, 3159 (1973).
- 11 G. S. Stein, M. Roberts, L. Davis, J. Head, J. Stein, L. Trall, J. Van Veen and W. Welch, *Nature* 258, 639 (1975).
- 12 C. Yeoman, J. Jordan, R. K. Busch, W. Taylor, E. Savage and H. Busch, *Proc. nat. Acad. Sci. USA* 73, 3258 (1976).
- 13 Lj. Ševaljević and M. Konstantinović, *Biochimie*, in press (1978).

## Tsetse fly reactions to light and humidity gradients

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**Summary.** Tsetse flies are positively phototactic below about 30°C and negatively phototactic above it. The flies show a preference for the wet end of a humidity gradient and the bright end of a dorsal light intensity gradient. Studies of activity levels indicate that tsetse flies should aggregate in damp situations where the activity level is minimal, whereas in practice the flies are distributed throughout the whole of a gradient. Analyses of the water and fat content of experimental flies indicates that the reactions of individual flies is determined by their physiological condition and the conditions under which the flies have previously been kept. Previous ecological studies on the reactions of flies to humidity and light stimuli need to be reassessed in the light of these findings.

Buxton and Lewis<sup>2</sup> showed that temperatures are minimal and humidity maximal at dawn in northern Nigerian thickets. From dawn to about mid-day the temperature rises and the relative humidity falls, after which the temperature remains constant, and the humidity rises slowly. After dusk, the temperature declines and the humidity rises until dawn of the following day, when the cycle is repeated. Under natural conditions a dorsal light intensity gradient develops at the junction of the riverine vegetation and adjacent savannah woodland or at the margins of the *Isoberrina doka* ecotone in northern Nigeria. Between dawn and noon, a humidity gradient was also observed to develop in these situations. The experimental investigations described below attempt to assess the reactions of *Glossina morsitans* and *G. austeni* to such diurnal environmental changes.

**Materials and methods.** The flies used in the experiments were reared from pupae of *G. morsitans* Westwood and *G. austeni* Newstead supplied by the Tsetse Fly Laboratories of the University of Bristol.

The reactions of tsetse flies to light intensity gradients. The reactions of tsetse flies to light intensity gradients were investigated using 17 specimens of *G. morsitans* in an artificial dorsal light gradient 120 cm long ranging from 1250 to 130 lux at 25°C. The experiments were performed with a

relative humidity (RH) of 100% and then repeated with a fresh batch of flies at 50% RH. The results are given in table 1.

The reactions of tsetse flies to a humidity gradient. Both sexes of teneral specimens of *G. morsitans* were placed in a 120 cm long humidity gradient ranging from 100 to 20% RH at 25°C under an illumination of 210 lux. 3 series of experiments were performed. In the first, the tsetse flies were kept in a humidity of 100% 48 h before the start of the experiment, and in the second series at 50%. In the third series *Calliphora erythrocephala* (Meigen) previously kept at 80% RH for 48 h were used. In a fourth series 12 gorged specimens of *G. austeni* were placed in a circular humidity gradient ranging from 0 to 100% RH and the positions of the flies recorded at 3-min intervals until 120 records had been obtained. The experiment was then repeated after 24 and after 48 h. The results are given in table 2 and in the text.

Measurements of activity levels. The activity levels of teneral male and female flies of *G. morsitans* were determined by placing the flies in a geigy cage at 25°C in an illumination of 210 lux in an atmosphere of either 20% or 100% RH. The number of spontaneous flights or walks per fly per min was then determined. The results are given in the text.

Table 1. Reactions of *Glossina morsitans* to a dorsal light intensity gradient

Conditions of experiment	Number of flies used	Number of positions recorded	Number of positions for each zone										Average resting position	$\chi^2$
			1	2	3	4	5	6	7	8	9	10		
Teneral females (humid atmosphere)	17	167	28	35	47	7	14	5	18	2	7	4	3.63	$p < 0.001$
Teneral males (humid atmosphere)	12	142	37	31	22	9	12	7	5	1	16	2	3.55	$p < 0.001$
Teneral females (arid atmosphere)	13	158	32	37	36	7	8	10	17	4	2	5	3.54	$p < 0.001$
Teneral males (arid atmosphere)	11	129	51	22	31	21	1	3	0	0	0	0	2.27	$p < 0.001$

Zone 1 is the highest light intensity, zone 10 the lowest.

$\chi^2$  test applied to single series of values of each experiment with an assumed uniform distribution for the controls.

Table 2. Reactions of *G. morsitans* and *Calliphora erythrocephala* to humidity gradients

Conditions of experiment	Number of flies	Number of positions recorded	Number of positions recorded in each zone					Average resting position	$\chi^2$	
			1	2	3	4	5			
<i>G. morsitans</i>										
Teneral females										
Experiment	14	588	141	52	50	29	316	3.6	}	p<0.001
Control	14	541	86	92	103	131	129	3.2		
Teneral males										
Experiment	20	488	117	48	42	55	226	3.3	}	p<0.001
Control	-	-	-	-	-	-	-	-		
Teneral males previously kept in 50% RH										
Experiment	18	648	46	88	147	156	211	3.6	}	p<0.001
Control	18	625	145	67	86	105	222	3.3		
Teneral males without antennae										
Experiment	9	448	100	53	120	84	91	3.0	}	p<0.001
Control	10	498	84	55	84	146	129	3.4		
Teneral females without antennae										
Experiment	18	541	26	78	148	109	180	3.6	}	p<0.001
Control	25	730	119	63	81	113	354	3.7		
<i>C. erythrocephala</i>										
Unfed males										
Experiment	7	169	10	2	17	42	98	4.3	}	p<0.001
Control	7	150	48	24	26	22	30	2.7		

Zone 1: dry, zone 5: moist.

$\chi^2$  test applied to experimental data and controls, except for experiment with teneral males, where single series of values of experiment used with an assumed uniform distribution for the controls.

Estimates of water and fat content. Estimates of water and fat content of the flies were determined using the standard techniques. The results are given in the text.

**Discussion of results.** Table 1 shows that whether the flies have been previously kept in a dry or a moist atmosphere they were distributed throughout the whole of the light-intensity gradient but showed a statistical preference for the brighter illuminations. These results may explain why tsetse flies aggregate near the tops of trees on moonlight nights and the presence of teneral flies in more open situations after dawn and before dusk in search of suitable hosts. Jacks<sup>3</sup> observations that tsetse flies are normally positively phototactic below about 30°C but negatively phototactic above this temperature have been confirmed and explain the occurrence of tsetse flies in various crevices and sheltered microhabitats at mid-day.

Table 2 shows that both sexes of *G. morsitans* were distributed throughout the whole of the humidity gradient, but showed a distinct statistical preference for the wet end of the gradient whether they had been previously kept at 50 or

100% RH. Although flies tended to aggregate at the tube ends, the experimental results are statistically different from those of the controls.

Lewis<sup>4</sup> has shown that the flagellum of tsetse flies has humidity receptor sensilla. Table 2 also shows that flies without antennae preferred drier conditions than the controls and suggests that the flagella are used to perceive humidity differentials. Table 2 also shows that, unlike *G. morsitans*, *Calliphora erythrocephala* shows a comparatively uniform response with nearly 60% of the flies going to the wet end of the gradient. The reactions of *G. austeni* in the circular humidity gradient showed that newly gorged flies settled 87 and 33 times in the dry and moist halves, respectively, 55 and 65, respectively, after 24 h and 31 and 89 times, respectively, after 48 h. These results show that the flies prefer dry conditions immediately after feeding and a moist atmosphere 48 h later when they are hungry. The levels of activity of *G. morsitans* were initially the same for both sexes, whether they were kept at 20% or 100% RH but after 24 h were 0.28 and 0.20 for teneral male and

female flies, respectively, at 20% RH and 0.009 and 0.03 for flies in an atmosphere with 100% RH. These results show that males were slightly more active than females, but that flies in damp conditions became less active than those in drier situations. Furthermore these differences in activity level suggest that flies should tend to aggregate in moist microhabitats where the degree of activity is least, whereas in fact they tend to be distributed throughout the whole of a gradient and also show slight statistical preferences for the wet end of the gradient. These apparent contradictions can be resolved if it is assumed that the reactions of individual flies depend upon their physiological condition, which in turn is determined by the conditions under which the flies have previously been kept. Further work showed that flies kept at 20% and 100% RH under similar conditions, initially had the same water content, but after 48 h the flies kept at the lower humidity had a lower water content than the flies kept in moist air; while the fat content of flies kept at a RH of 20% ranged from 2.6 to 39.1% (a range of 36.5%) of the b.wt compared with a range of 15.8 to 26.1% (i.e. a range of 15.8%) for the flies kept in a moist atmosphere. Buxton and Lewis<sup>2</sup> found that tsetse flies kept in a low humidity metabolize fats to maintain their water content. These experiments show that *G. morsitans* loses water and metabolizes more fat when kept in low humidities and that these physiological changes are asso-

ciated with a rise in the level of activity, which would initially cause them to move away from existing conditions. Over a period, these various factors may encourage flies to aggregate in moist situations, where the activity level is minimal, but they are probably also responsible for producing a wide range of reactions within a given fly population. Hungry flies are attracted to mammalian hosts by their form and smell<sup>5</sup> radiant heat<sup>6</sup>. The above experiments show that gorged flies are initially attracted to dry microhabitats and then show an increased tendency to settle in moister situations.

These experiments show that the reactions of *G. morsitans* to humidity and light gradient are more complex than has previously been supposed and that the validity of previous ecological investigations needs to be reassessed in the light of these findings.

- 1 Present address: St. Martin's College of Education, Lancaster, Lancashire, England.
- 2 P.A. Buxton and D.J. Lewis, Phil. Trans. R. Soc. (B) 224, 175 (1934).
- 3 R.W. Jack, Sth. Rhod. Mem. Dept. Agr. 1, 1 (1939).
- 4 C.T. Lewis, Symp. R. ent. Soc. Lond. 5, 59 (1970).
- 5 C.F.M. Swynnerton, Trans. R. ent. Soc. Lond. 84, 1 (1936).
- 6 M. Abdillahi, thesis, University of Salford (1974).

## Prostaglandin-like substances in *Propionibacterium acnes* II. Stimulatory effect on ovarian cyclic AMP

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**Summary.** The prostaglandin-like substances (PLS) from *Propionibacterium acnes* increased the ovarian tissue levels of cyclic AMP (cAMP) approximately 2-fold. The lipid material extracted from *P. acnes* thus behaved like PG's of the E-type, and since it is unlikely that other known stimulators of the ovarian cAMP system can be present in the bacterial lipid fraction, these experiments give further evidence in favour of the occurrence of PLS in *P. acnes*.

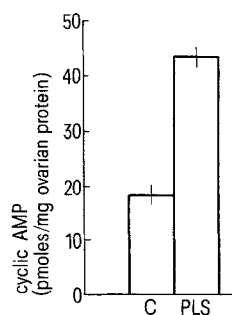
*P. acnes* is acknowledged to play a pivotal role in the inflammatory reactions in acne vulgaris. On studying lipids of *P. acnes*, we discovered the occurrence of prostaglandin-like substances (PLS)<sup>1,2</sup>. The bacteria were cultured on chemically defined media, carefully extracted and the lipid fraction was further purified by several chromatographic procedures. The combined gas chromatographic-mass spectrometric analysis is now in progress. However, to investigate the biological properties of these substances, bioassays were performed on smooth muscle of the utero-tubal junction (UTJ), as well as on gerbil colon. The UTJ bioassay demonstrated that these compounds possessed a prostaglandin-like effect on the spontaneous contractility of the smooth muscle strips<sup>3</sup>. In the gerbil colon bioassay contractile effect was recorded<sup>4</sup>. Thus, in both systems, the PLS of *P. acnes* mimics prostaglandins of the E-type (PGE). Lately, these compounds has been shown to elicit the PGE-like response in hamster pouch vessels<sup>5</sup>.

It is well-known that PGE are potent stimulators of the cyclic AMP (cAMP) system in the prepubertal rat ovary (for references see Selstam et al.<sup>6</sup>). To characterize more closely these new substances extracted from *P. acnes*, their effect on the ovarian cAMP system was studied.

**Animals.** Rats of the Sprague-Dawley strain, 23 days old, obtained from Anticimex Ltd, were used. They were deprived of food 24 h before the experiments, but allowed to drink tap water ad libitum.

**Chemicals.** Tritiated cAMP (<sup>3</sup>H-cAMP) with a specific activity of 31 Ci/mmol was purchased from New England Nuclear Co., Boston, Mass., USA. Cyclic 3', 5'-AMP-dependent protein kinase and protein kinase inhibitor for the cAMP assay were obtained from Sigma Ltd. All other chemicals were of analytical grade and purchased from Merck Co. or Sigma Ltd.

**Experimental procedures.** 4 rats were sacrificed by cervical fractures and the ovaries were rapidly removed and placed in ice-chilled buffer. Each ovary was trimmed free from extraneous tissues, rinsed, blotted on a filter paper, weighed (range: 6.9–9.1 mg) and put into a 'preincubation' flask. The preincubation period lasted for 60 min in a medium



Effect of prostaglandin-like substances (PLS) from *P. acnes* on the cAMP content in the prepubertal rat ovary when compared with control (C). For details see the text.