mucosal vasodilatation[14], however it increases stomach contractions[15] which can lead to mucosal vascular engorgement[11]; both these effects are reflected by elevated microcirculatory blood volumes. Further investigations are therefore necessary to establish quantitatively their contributions to the increased microcirculatory volume, since blockade of either or both of these H_1- receptor effects could have been responsible for the observed action with mepyramine. The ability of H_2-receptor block to inhibit stress-induced mucosal vasodilatation is equally interesting, and may explain the antiulcer action of metiamide[3] and of doses of metiamide[16] or cimetidine[17] which do not influence gastric acid secretion. H_2-receptor stimulation in the stomach has so far been shown only to dilate mucosal microvessels[14,18], and to increase acid secretion[15] which does not contribute to stress ulceration in rats[19,20]. Mucosal vasodilatation is likely to be due to a direct vascular effect of released gastric histamine, and not secondary to increased histamine-mediated acid secretion[18], because the antiulcer action of H_2-receptor blockade occurs even when acid secretion is unaffected[16,17].

1 Acknowledgment. The authors thank Dr W.A.M. Duncan (Smith, Kline and French Labs Ltd, England) for the generous gift of metiamide.

2 C.H. Cho and C.W. Ogle, Proc. 12th Singapore-Malaysia Congr. Med., in press.
3 S. Dai, C.W. Ogle and C.H. Lo, Eur. J. Pharmac. *33*, 277 (1975).
4 S. Bonfils and A. Lambling, in: Pathophysiology of Peptic Ulcer, p. 162. Ed. S.C. Skoryna. McGill University Press, Montreal 1963.
5 D.A. Brodie, Gastroenterology *43*, 107 (1962).
6 C.H. Cho and C.W. Ogle, Eur. J. Pharmac. *43*, 315 (1977).
7 C.H. Cho and C.W. Ogle, Eur. J. Pharmac. *48*, 97 (1978).
8 S. Dai and C.W. Ogle, Eur. J. Pharmac. *30*, 86 (1975).
9 P.H. Guth and P. Hall, Gastroenterology *50*, 562 (1966).
10 T. Räsänen, in: Peptic Ulcer, p. 237. Ed. C.J. Pfeiffer. Munksgaard, Copenhagen 1971.
11 J.C. Schwartz, in: Peptic Ulcer, p. 190. Ed. C.J. Pfeiffer. Munksgaard, Copenhagen 1971.
12 T. Räsänen, Gastroenterology *38*, 70 (1960).
13 C.W. Ogle and C.H. Cho, Pharmac. Res. Commun. *9*, 679 (1977).
14 I.H.M. Main and B.J.R. Whittle, J. Physiol., Lond. *246*, 33P (1975).
15 J.W. Black, W.A.M. Duncan, C.J. Durant, C.R. Ganellin and E.M. Parsons, Nature, Lond. *236*, 385 (1972).
16 J. Bugajski, J. Hano and L. Danek, Eur. J. Pharmac. *36*, 237 (1976).
17 S. Okabe, K. Takeuchi, T. Urushidani and K. Takagi, Am. J. dig. Dis. *22*, 677 (1977).
18 B.M. Johnston and D.A.A. Owen, Eur. J. Pharmac. *44*, 355 (1977).
19 S. Dai and C.W. Ogle, Eur. J. Pharmac. *26*, 15 (1974).
20 K. Takagi and S. Okabe, Eur. J. Pharmac. *10*, 378 (1970).

Cyclic AMP is a likely mediator of ovulation in the tsetse fly[1]

D.L. Denlinger, M.F.B. Chaudhury and T.S. Dhadialla

Department of Entomology, Ohio State University, Columbus (Ohio 43210, USA) and International Centre of Insect Physiology and Ecology, P.O. Box 30772, Nairobi (Kenya), 13 March 1978

Summary. Ovulation in tsetse flies is normally induced by mating, but virgins can be stimulated to ovulate with an injection of dibutyryl cyclic AMP, cholera toxin (a cyclic AMP generator), or aminophylline (a phosphodiesterase inhibitor). Thus, elevation of cyclic AMP is a likely link in the events leading to ovulation.

Virgin females of many insect species produce mature eggs within their ovaries, but seldom are the eggs ovulated unless she has an opportunity to mate[2,3]. Such is the case in the tsetse fly, the vector of African sleeping sickness[4,5]. Successful mating is a prerequisite for ovulation, and the single egg matured in the ovary of a virgin tsetse is eventually resorbed if the female remains unmated. The act of mating triggers ovulation by stimulating the female's brain to release a neurosecretion that is conveyed by the blood to its target organ, the ovary[6]. A follicular plug at the base of the ovary ruptures and contractions of the oviduct propel the egg into the uterus. We suggest that cyclic AMP is a likely mediator of ovulation in tsetse flies since we find that ovulation can be induced in virgin females with dibutyryl cyclic AMP and other chemical agents that elevate levels of cyclic AMP.

Test compounds (cholera toxin was obtained from Schwarz/Mann and all other chemicals were from Sigma) were injected without anaesthesia into 12-day-old virgin female *Glossina morsitans morsitans* Westw. through the dorsal region of the thorax using a finely drawn, calibrated glass capillary. Flies of this age were selected to optimize the probability that the ovary would contain an egg that was fully mature but had not yet begun to be resorbed[6]. The incidence of ovulation was determined by dissecting the flies 24 h after treatment.

The 1st line of evidence implicating a role for cyclic AMP in tsetse ovulation comes from the effect elicited by an injection of dibutyryl cyclic AMP. As shown in the table, dibutyryl cyclic AMP (N^6, $O^{2'}$-dibutyryl adenosine 3′:5′-cyclic monophosphoric acid) caused 55% of the virgin females to ovulate. Cyclic AMP (adenosine 3′:5′-cyclic monophosphoric acid) and 8-bromo-cyclic GMP (8-bromoguanosine 3′:5′-cyclic monophosphoric acid) were ineffective. In several other organisms, dibutyryl cyclic AMP has also been found to be a more potent agent than cyclic

Effect of cyclic nucleotides, aminophylline, and cholera toxin on stimulating ovulation in 12-day-old virgin females of *Glossina morsitans*

Material injected	Number	Ovulation (%)
Distilled water (1 µl)	22	5
8-bromo cyclic GMP (25 µg)	18	0
Cyclic AMP (25 µg)	19	0
Dibutyryl cyclic AMP (25 µg)	22	55
Aminophylline (10 µg)	20	45
Aminophylline (10 µg) + dibutyryl cyclic AMP (25 µg)	17	53
Cholera toxin (0.5 µg)	21	33

An injection volume of 0.5 µl was used for cholera toxin and 1.0 µl for all other chemicals.

AMP[7,8], presumably due to either its superior ability to penetrate the cell membrane or greater resistance to inactivation by phosphodiesterase. That 1 water-injected virgin did ovulate suggests the requirement for mating to precede ovulation[4,5] is not entirely inviolable.

By preventing the degradation of cyclic nucleotides, a phosphodiesterase inhibitor such as aminophylline (theophylline$_2$-ethylenediamine) should elevate endogenous levels of cyclic AMP, and thus be a likely agent to mimic the effect of a cyclic AMP injection. Indeed, ovulation was induced in 45% of the females injected with 10 µg aminophylline (table). A simultaneous injection of aminophylline and dibutyryl cyclic AMP, however, did not elevate the response beyond that observed with dibutyryl cyclic AMP alone.

A role for cyclic AMP in tsetse ovulation is also suggested by the effectiveness of cholera toxin. Cholera toxin, a potent stimulant of adenylate cyclase in insects[9] as well as vertebrates[10], caused 33% of the virgin females to ovulate within the 24-h test period (table).

Thus, 3 lines of evidence suggest an important role for cyclic AMP in tsetse ovulation. We cannot yet eliminate the possibility that cyclic AMP exerts its effect by stimulating the release of ovulation hormone from the brain, but it is quite likely that we have bypassed the release of ovulation hormone and have mimicked the effect of the hormone directly on the target tissue. Like many other neurohormones[11-13], the ovulation hormone possibly uses cyclic AMP as a 2nd messenger in triggering its response within the ovary.

In most insects ovulation is followed immediately by oviposition, but the tsetse female has evolved a unique reproductive strategy in which the fertilized egg and resulting larva are retained within her uterus[14,15]. Nutriment is channeled to the larva from a female accessory gland that has been highly modified for 'milk' production[16]. At intervals of about 9 days, the female gives birth to a fully grown 3rd instar larva that immediately burrows into the soil and pupariates. In a normal 9-day pregnancy cycle, parturition and ovulation occur closely together: within 1 h of expulsion of the larva, a new egg is ovulated[14,15]. Similar endocrine events possibly stimulate the muscular contractions responsible for both ovulation and parturition. In several virgin females, eggs were not only ovulated but also expelled from the uterus following injection of either cholera toxin or dibutyryl cyclic AMP. Moreover, pregnant females injected with 0.5 µg cholera toxin during the 2nd pregnancy cycle had a high incidence of premature parturition: 57% of the females (N = 21) aborted within 2 days. An injection of 25 µg dibutyryl cyclic AMP caused 37% abortion (N = 16). Among the controls injected with 1 µl distilled water, only 5% of the pregnant females (N = 18) aborted within 2 days. Our preliminary evidence thus suggests that elevation of cyclic AMP levels may be involved in tsetse parturition as well as ovulation.

1 We thank J. de Wilde and W.A. Foster for helpful comments. D.L.D.'s research visit to ICIPE was supported by a grant from the Mid-West Universities Consortium for International Activities.

2 F. Engelmann, The Physiology of Insect Reproduction. Pergamon Press, Oxford 1970.
3 J. de Wilde and A. de Loof, in: The Physiology of Insecta, vol. 1, p. 142. Ed. M. Rockstein. Academic Press, New York 1973.
4 T.R. Odhiambo, J. exp. Zool. *177*, 447 (1971).
5 D.S. Saunders and C.W.S. Dodd, J. Insect Physiol. *18*, 187 (1972).
6 M.F.B. Chaudhury and T.S. Dhadialla, Nature *260*, 243 (1976).
7 R.W. Butcher, G.A. Robison and E.W. Sutherland, in: Biochemical Actions of Hormones, p. 21. Ed. G. Litwack. Academic Press, New York 1972.
8 Th. Posternak, in: Cyclic AMP, p. 48. Ed. G.A. Robison, R.W. Butcher and E.W. Sutherland. Academic Press, New York 1971.
9 D.L. Denlinger, in: Molecular Biology and Pharmacology of Cyclic Nucleotides, p. 319. Ed. G. Folco and R. Paoletti. Elsevier, Amsterdam 1978.
10 R.A. Finkelstein, CRC Crit. Rev. Microbiol. *2*, 553 (1973).
11 B.J. Cook, G.M. Holman and E.P. Marks, J. Insect Physiol. *21*, 1807 (1975).
12 G. Fraenkel, A. Blechl, J. Blechl, P. Herman and M.I. Seligman, Proc. nat. Acad. Sci., USA *74*, 2182 (1977).
13 J.W. Truman, A.M. Fallon and G.R. Wyatt, Science *194*, 1432 (1976).
14 D.S. Saunders and R.J. Phelps, in: The African Trypanosomiases, p. 327. Ed. H.W. Mulligan. Wiley, New York 1970.
15 D.L. Denlinger and W.-C. Ma, J. Insect Physiol. *20*, 1015 (1974).
16 W.-C. Ma and D.L. Denlinger, Nature *247*, 301 (1974).

Hemolymph regulation to hyposaline and hypersaline conditions in *Gammarus oceanicus* (Crustacea: Amphipoda)

D.A. Brodie[1] and K. Halcrow[2]

Department of Biology, University of New Brunswick, Saint John (New Brunswick, Canada E2L 4L5), 13 February 1978

Summary. Specimens of *Gammarus oceanicus* were exposed to sea water at salinities of 3‰ and 55‰ for various periods of time up to 24 h. *G. oceanicus* can regulate in hyposaline sea water and reached a new steady state after 4 h exposure to 3‰ sea water. Its ability to regulated in hypersaline conditions is limited and no animals survived longer than 6–8 h in 55‰ sea water. These results are discussed with respect to salinity conditions in the intertidal zone.

In a recent study, it was necessary to obtain hemolymph osmolality values for *Gammarus oceanicus* exposed to hyposaline and hypersaline conditions for various lengths of time. *G. oceanicus* is the most abundant *Gammarus* species and often the most numerous intertidal macroscopic animal between the northern Gulf of Maine and Newfoundland[3], but its ability to osmoregulate is poorly understood. *G. oceanicus* has been exposed to various hyposaline conditions for 24 h and its hemolymph molality recorded[4]. The present study reports values for hemolymph osmolality in animals exposed to hyposaline and hypersaline conditions for short time intervals.

Materials and methods. Male *Gammarus oceanicus,* larger than 20 mm in length from the rostrum to the base of the telson were exposed to either 3‰ diluted sea water or 55‰ 'Instant Ocean' salts in distilled water at a temperature of 12–13 °C. They were initially collected from 32‰ sea water. The animals were exposed for periods of 10 min, 30 min,