on ^{45}Ca release from the pancreas nor did it alter the response to carbachol. The dibutyryl analogue of cyclic GMP did not duplicate or alter the inhibitory effect of carbachol on ^{3}H-leucine incorporation into pancreatic trichloroacetic acid-precipitable protein.

Résumé. L'effet du dibutyryl GMPc sur la sécrétion d'α-amylase, l'efflux du ^{45}Ca et la synthèse de nouvelles protéines dans le pancréas du rat, ont été étudiés. Le dibutyryl GMPc n'affecte ni la sécrétion d'enzyme basale ni celle stimulée par le carbachol. Le nucléotide n'a pas un effet significatif sur le relâchement du ^{45}Ca du pancréas et n'altère pas la réponse du carbachol. L'analogue ne mime pas et ne change pas l'effet du carbachol sur l'incorporation du ^{3}H-leucine dans les protéines précipitées par l'acide trichloroacétique.

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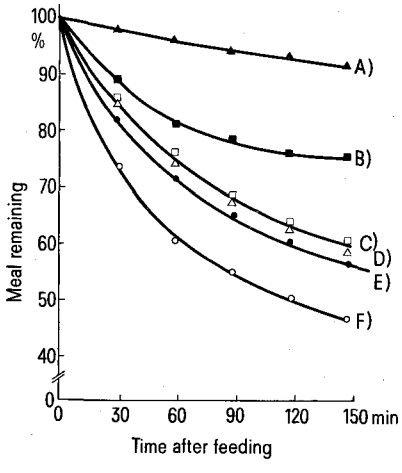
Inhibition of Diuresis in the Tsetse Fly (*Glossina morsitans*) by Ouabain and Acetazolamide

Recently TOBE[1] reviewed the literature dealing with diuresis following a blood meal in tsetse flies and presented evidence concerning water movements in female *Glossina austeni*. TOBE showed that $^{3}H_2O$ was rapidly eliminated after it had been ingested in a meal of defibrinated beef blood and that the specific activity in the urine was approximately the same as the specific activity in the meal; the specific activity of tsetse haemolymph was, however, considerably lower than the meal on which the flies fed. By feeding ^{14}C-carboxyl dextran in defibrinated beef blood, TOBE showed that 13 of 31 flies excreted significant amounts of ^{14}C within 5 min of feeding, but no ^{14}C was found in the haemolymph. TOBE proposed 2 models to explain the results: in the first model water passes directly down the gut and in the second model water passes through the gut wall and Malpighian tubules and there is a compartmentalization of the tsetse haemolymph. The evidence supported both models and it was not possible to choose between them. I therefore wish to report my observation that both acetazolamide and ouabain, metabolic inhibitors which interfere with certain membrane transport systems, reduce the rate of water elimination by male *Glossina morsitans morsitans*.

Materials and methods. G. morsitans used in this experiment were from our colony which is maintained by feeding on rabbits 6 days per week[2]. Previously unfed males were individually weighed (to 0.01 mg) when 24 to 48 h old, fed the experimental meal through an Agar/Parafilm membrane[3], held at ca. 22 °C and reweighed at 30 min intervals. The meal consisted of 0.85% NaCl, 1 m*M* ATP (adjusted to pH 7.4) with or without ouabain or acetazolamide.

Results and discussion. The 62 flies used in this experiment weighed an average of 16.41 ± 2.43 mg and consumed meals averaging 24.87 ± 5.34 mg. There was no significant difference between the meal size of flies fed upon saline and those fed upon saline plus ouabain or acetazolamide. The data in the Figure show a rapid loss of weight in *G. morsitans* males fed upon a saline solution. The excretion rate of the controls is comparable to the rate reported for *G. morsitans* males fed heparinized goat blood or haemolyzed bovine blood[4]. Both of the metabolic inhibitors ouabain and acetazolamide reduce the rate of water loss. Ouabain is an inhibitor which, in most animals, interferes with a Na^+/K^+ active transport system across membranes. Acetazolamide is a specific inhibitor of carbonic anhydrase and as such also interferes with ion and water transport across membranes[5]. Evidence for ouabain sensitive transport mechanisms in insects has been obtained with *Periplaneta americana*[6] and *Drosophila hydei*[7,8] but not *Rhodnius prolixus*[9] or *Carausius morosus*[10]. On the basis of the effects of Na^+ and K^+ upon water excretion LANGLEY and PIMLEY[4] suggested that an active transport system exists in *G. morsitans*. Carbonic anhydrase has been demonstrated in several tissues of a number



Effect of acetazolamide and ouabain on diuresis by male *G. morsitans* after feeding on a meal of 0.85% NaCl, 1 m*M* ATP at pH 7.4. Each point is the mean of 8 to 12 determinations; the number of flies used is given in parentheses. The standard deviation for each of the means was approximately 10% of that mean.
A) 300 μg/ml acetazolamide (12); B) 20 μg/ml ouabain (10); C) 0.2 μg/ml ouabain (8); D) 50 μg/ml acetazolamide (11); E) 0.2 μg/ml ouabain plus 50 μg/ml acetazolamide (11); F) control (10).

[1] S. S. TOBE, Experientia *30*, 517 (1974).
[2] This colony was started with pupae obtained in July 1973 from the Tsetse Research Laboratory, University of Bristol, Langford, Bristol, England. Flies in our colony are comparable to the Langford colony in terms of pupal weight, longevity, and reproductive capacity.
[3] The system used consisted of a 1 mm thick slab of agar laid over a stretched parafilm membrane as described by P. A. LANGLEY, Bull. ent. Res. *62*, 215–228, and further modified by A. MEWS (unpublished) by using a stippled glass plate to hold the artificial meal and by adding 2% glycerol to the agar during its preparation.
[4] P. A. LANGLEY and R. W. PIMLEY, J. Insect Physiol. *19*, 1097 (1973).
[5] P. W. HOCHACHKA and G. N. SOMERO, *Strategies of Biochemical Adaptation* (W. B. Saunders Co. Philadelphia, London, Toronto 1973), p. 358.
[6] A. M. O'RIORDAN, J. exp. Biol. *51*, 699 (1969).
[7] E. WEBER-VON GROTTHUSS, F. HEVERT, U. ATZBACHER and A. WESSING, J. Insect Physiol. *20*, 1411 (1974).
[8] U. ATZBACHER, F. HEVET, E. WEBER-VON GROTTHUSS and A. WESSING, J. Insect Physiol. *20*, 1989 (1974).
[9] S. H. P. MADDRELL, J. exp. Biol. *51*, 71 (1969).
[10] D. E. M. PILCHER, J. exp. Biol. *53*, 465 (1970).

of insects (summarized by EDWARDS and PATTON[11]) and it is sensitive to acetazolamide.

Ouabain and acetazolamide when administered together (Figure E) were no more effective than when administered alone (Figure C and D). This may be explained in one of several ways. Ouabain and acetazolamide may inhibit the same enzyme in *G. morsitans*, but this seems very unlikely because of the specificity of these inhibitors in other organisms. It is also possible that one of the inhibitors is blocking the absorption of the other so that no additive or synergistic effect can be observed. The most intriguing possibility is that the ouabain-sensitive Na^+K^+ATPase and the acetazolamide-sensitive carbonic anhydrase influence ion and water transport across the same membrane with H^+ serving as the counterion during transport of Na^+.

If ouabain and acetazolamide function in *G. morsitans* in the same way as they function in other animals then my results suggest that water excretion by tsetse flies involves transport across membranes. If this is true also for female *G. austeni* it suggests that the most likely explanation of TOBE's results is the compartmentalization model he proposed[1].

Summary. Acetazolamide and ouabain, metabolic inhibitors which interfere with certain membrane transport systems, reduce the rate of water elimination by male *Glossina morsitans morsitans*. The results suggest that water is transported across membranes during diuresis and that a ouabain sensitive Na^+K^+ATPase and an acetazolamide-sensitive carbonic anhydrase are involved in diuresis.

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[11] L. J. EDWARDS and R. L. PATTON, J. Insect Physiol. *13*, 1333 (1967).

[12] I thank Miss M. I. BUICK for her assistance in maintaining the tsetse colony, Mr. B. M. ROLSETH for his assistance in this experiment and Dr. D. A. CRAIG and Dr. B. K. MITCHELL for their comments on the manuscript. This work was financed (in part) by a grant (No. 6801–51) from the Defence Research Board of Canada.

Seasonal Variations in Mitosis in the Frog: A Field Study[1]

Our laboratory has recently obtained evidence suggesting seasonal variation in mitotic activity of the frog (*Rana pipiens, Rana catesbeiana*) lens which is mediated by anterior pituitary factors. The work was performed shortly after the experimental animals were secured from commercial supply houses. It was impossible to be certain of the conditions to which they were subjected enroute to the laboratory. Furthermore, suppliers distribute various races and even subspecies of *Rana pipiens* without differentiating among them. In order to overcome the uncertainties in interpretation of data caused by these factors, we undertook a field study in which all frogs were purchased from a single supplier located here in Vermont (Lake Champlain Frog Farm, Alburg, Vermont). The results of this study are reported here.

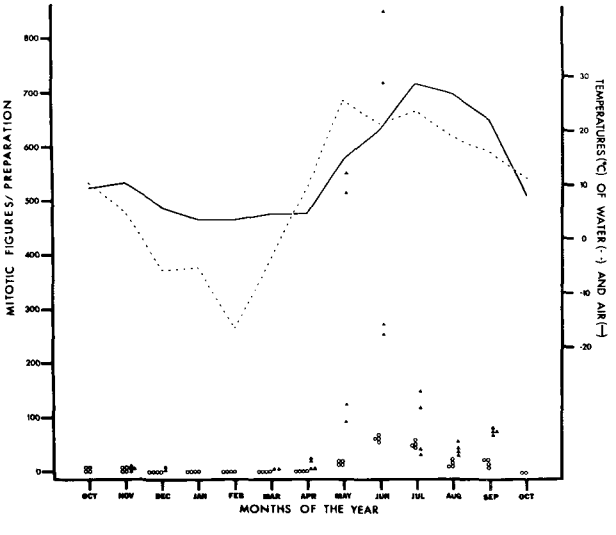

Fig. 1. Fluctuations in lens (○) and corneal (▲) mitoses through the year. The number of division figures in the tissues rises between April and May. From December until April, no mitoses are seen in lenses and their frequency in cornea is very low. The water temperatures presented were measured when collections were made. Air temperatures are averages (daily) obtained from the U.S. National Weather Service, Burlington, Vermont. Note that the peak of activity is reached in both cases during June and that there is a subsequent decline despite a continued rise in temperature.

Materials and methods. The animals all arrived in the same shipment and originated from a population indigenous to this vicinity. They were placed in a suitable enclosure in a pond in Shelburne, Vermont in September, 1973. Sampling commenced in October, 1973 and was carried through until October, 1974. Collections were made as close to the 15th of each month as possible. The frogs were sacrificed soon after capture. Lens and corneal epithelium were prepared for histological examination. Whole-mounts of the above tissues were scored for mitotic activity.

Samples of kidney, skin and lung were placed in Earle's Balanced Salt Solution for 5 min so that they could equilibrate with room temperature (24 ± 2 °C). They were then exposed to ^{3}H-thymidine. The tissues were fixed and then sectioned for autoradiography.

During the fall the animals became torpid and were retrieved in this state from the pond. In winter months, they were secured by drilling through the ice cover with an auger. Temperatures of both water and air were recorded over the 13 month period.

In several experiments, hypophysectomies were performed. These were executed according to the procedure of HOGBEN[2]. Only the adenohypophysis was removed.

Results. Figure 1 shows that mitotic activity of the frog lens epithelium abates between October and April. The water temperature at this time varied from 4 °C–9 °C. Corneal epithelium was not evaluated in every month but showed extremely low mitotic activity during November, December, March and April. A noticeable increase in proliferation is seen in both tissues during May and June. The corneal epithelium shows a much larger response than does that of the lens.

[1] This work was supported by USPHS Grant No. EY 00281-11 from the National Eye Institute.