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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[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.

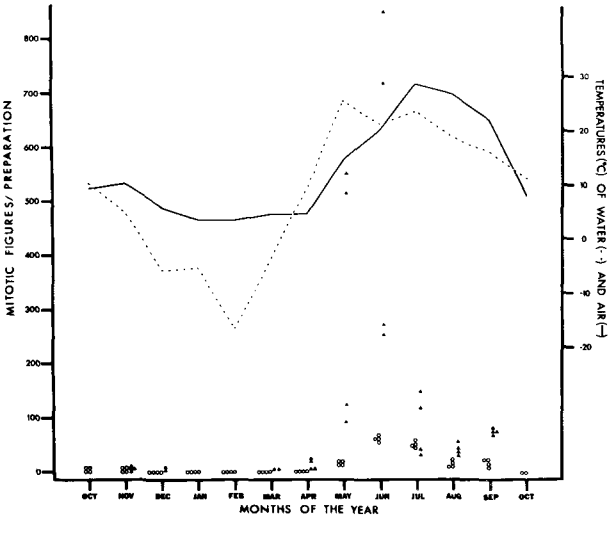

Fig. 1. Fluctuations in lens (○) and corneal (▲) mitoses through the year. The number of division figures in the tissues rises between April and May. From December until April, no mitoses are seen in lenses and their frequency in cornea is very low. The water temperatures presented were measured when 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.

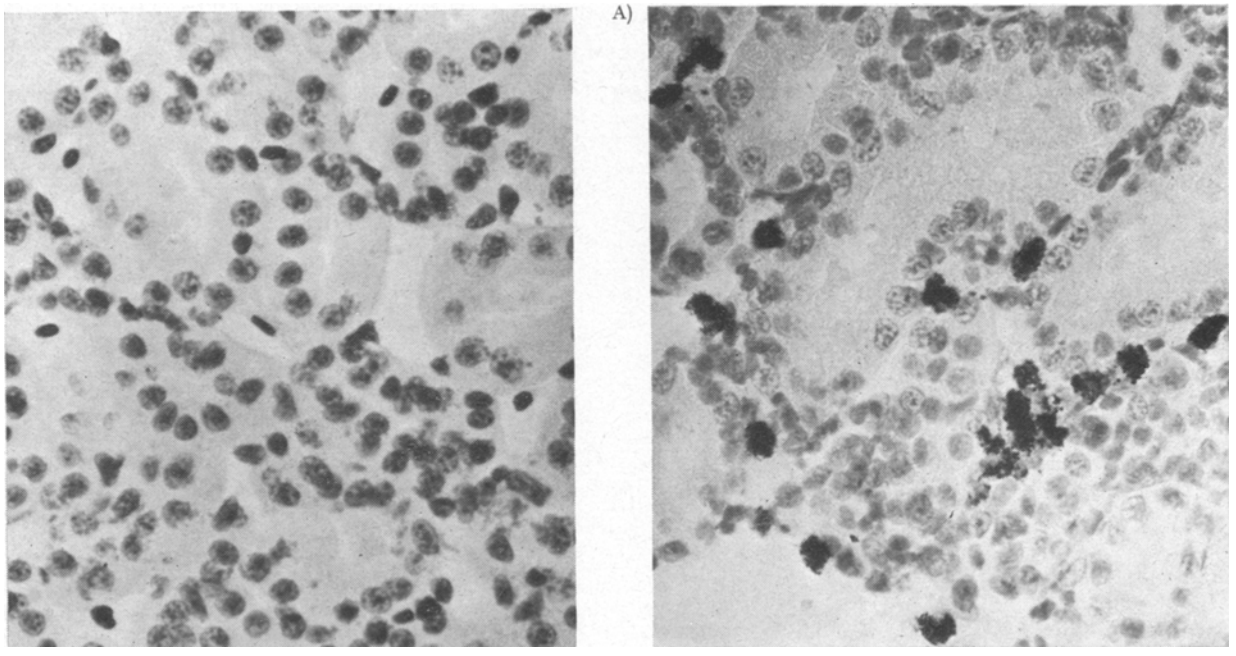

Fig. 2. Autoradiograms of kidney sections which originated from animals retrieved in November (B) and July (A). Both samples were incubated at 24 ± 2 °C in Eagle's minimal essential medium. Expolure to ^{3}H-thymidine 5 μCi/ml; Spec. Act. 6.4 Ci/m*M*) was for 1 h. ×500.

The other tissues studied seemed to behave as did the lens and cornea. There tended to be little or no DNA synthesis during the cold months and a variable but significant amount during the warm ones. Figures 2A and B show some typical specimens of renal tissues secured from animals during July and November.

One can obtain a response in the laboratory which is, in many respects, similar to that recorded in the seasonal study (Figure 3). It is of interest to note that hypophysectomy inhibits the mitotic rebound in lens but fails to interfere with the response of corneal epithelium. Sham hypophysectomy does not alter mitotic frequency.

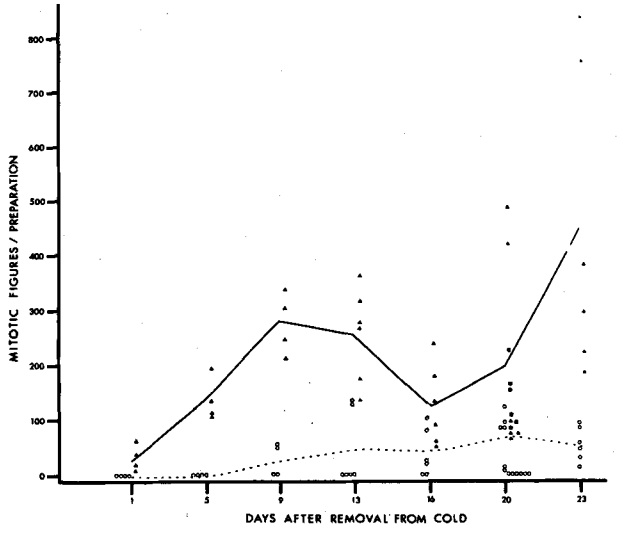

Fig. 3. The mitotic rebound in lens (○) and cornea (▲) which was obtained in the laboratory is illustrated. The animals were kept at 4 °C for 2 weeks. During this time mitosis disappears almost completely. The curves show reinitiation of proliferation after the frogs were placed at 24 ± 2 °C. Corneal activity resumed within the first day of transfer whereas 9 days were required for the lens. Hypophysectomy prevents the rebound in lens (□) but has no apparent effect on the corneal (■) response. Curves are drawn through the averages.

Discussion. Seasonal variations in lenticular mitotic activity have also been reported by SAKHAROVA and GOLITCHENKOV[3] for *Rana temporaria*. These authors observed relatively high rates of division during the warm part of the year and low ones during the cold months. Our view is that the increase in mitosis during spring (in *Rana pipiens*' lenses) is due to temperature-induced hypothalamic release with subsequent involvement of appropriate anterior pituitary hormones[4–6]. Anterior pituitary hormones from frogs have been obtained in pure form in our laboratory by gel electrophoresis. Of these, growth hormone and prolactin are able to stimulate DNA synthesis and mitosis in the lenses of hypophysectomized organisms[7,8].

Wound hyperplasia in frog lens epithelium is inhibited in hypophysectomized frogs and can be reinitiated at the injury site by means of replacement therapy[9]. Ongoing studies with injured corneal epithelium and endothelium suggest that tissue repair (via mitosis), in the latter but not the former, requires pituitary factors.

To what degree mitosis in extra-ocular tissues is influenced by pituitary factors we cannot say at this time. A more detailed report of these findings will appear presently.

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Summary. Field studies have shown that there is a seasonal variation in mitosis of lens and corneal epithelium (high in May and June, low during the rest of the year). This phenomenon can be reproduced in the laboratory by temperature manipulation. The response in the lens depends on the presence of the pituitary gland while the corneal one seems to be independent of it.

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Relationship Between Ca-ATPase Activity and Subunits of Myosin in the Myocardium of Rats Conditioned by Swimming

Native myosin of fast (F-myosin), slow (S-myosin) and cardiac (C-myosin) muscle prossesses ATPase activity. The ATPase activity of extracted and purified myosin in vitro can be activated in several ways, e.g. by Ca^{++}. This activity can be changed under normal circumstances or following chronic alterations of physiological state (development of organism[1], work overload[2,3], muscle dystrophy[4], heart failure[3,5]). It seems that the enzymatic activity of contractile proteins generally correlates with the functional and contractile capability of the respective muscle[1,6]. The mechanism by which the enzymatic activity of myosin is changed, as well as the reciprocal changes in the myosin molecule which enable this dynamic of the ATPase activity and force velocity relation, is of considerable theoretical interest and today is still unclear.

The aim of the present study was to determine whether changes in the specific Ca-ATPase activity in contractile proteins following work overload are linked causally with the changes in molecular weight of the light chains (LC) of myosin or the change in their proportions to each other.

The Ca-ATPase activities were studied in the cardiac actomyosin- and myosin solutions from control rats (CH) and of rats conditioned by swimming (SH). The duration of swimming (water temperature 35 °C) was 100–120 h in 8–10 weeks.

Actomyosin was extrated from fresh ventricular tissue using 3 vol. of Weber-Edsall-solution for 24 h at 4 °C and purified by repeated precipitation. The myosin was prepared by dissocisation of the above actomyosin by the ultracentrifugal separation of myosin and actin according to Weber[7]. After swimming training, Ca^{++}-ATPase activity in actomyosin- and myosinsolutions was significantly increased compared to the control rats. This held good for a large range of different Ca^{++}- and K^{+}-concentrations[2].

By SDS-gel electrophoresis[8] using 10% polyacrylamide, we found that cardiac myosins from both trained (SH) and untrained (CH) rats posses 2 light chains with a mol wt from 26,000 (LC_1) and 18,500 (LC_2). Differences in mol wt between 2 light chains of CH and of SH could be excluded.

However, the stoichometry of these chains indicated that the changes in the enzymatic activity of C-myosin of trained rats were accompanied by an altered quantitative relationship of both light chains of myosin. The relative amount of the electrophoretically slower component (LC_1) with a mol wt of 26,000 was significantly higher in myosin from SH than in the myosin from CH. Whereas the ratio

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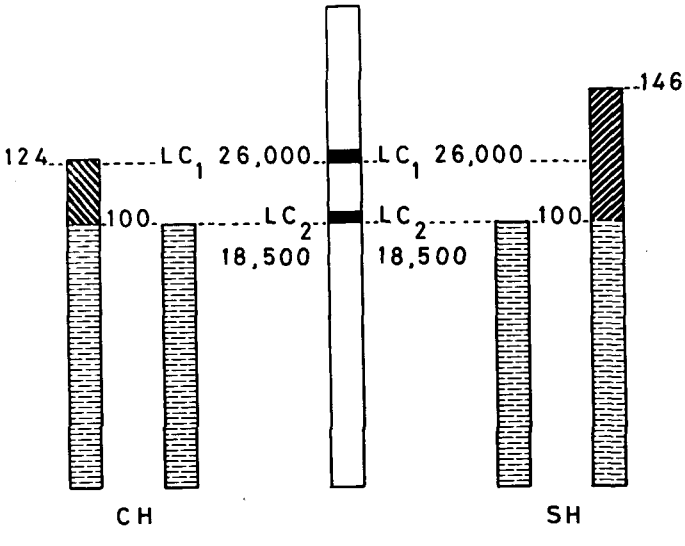

Fig. 1. Average shift in the relation LC_1/LC_2 in SH (146:100) in comparison with CH (124:100). SH, cardiac myosin of rats conditioned by swimming; CH, cardiac myosin of control rats.