Effects of daylength (hours of light per day LD) and temperature (°C) on plasma androgen concentration (expressed as ng androgen, testosterone and dihydrotestosterone, per ml of plasma) of male *Rana esculenta*

Experimental groups	Control	24 °C ± 1 °C LD 0:24	LD 24:0	LD 12:12	4 °C ± 1 °C LD 12:12
A January 1977*	6.56 ± 3.31	8.51 ± 1.88	18.66 ± 3.23**	-	4.34 ± 0.32
B June 1977	2.11 ± 0.29	2.41 ± 0.87	2.72 ± 0.93	1.69 ± 0.27	2.14 ± 0.64
C July 1977	1.61 ± 0.45	1.29 ± 0.43	1.21 ± 0.35	1.36 ± 0.46	1.21 ± 0.74
D October 1977	1.36 ± 0.42	2.05 ± 0.94	-	2.66 ± 1.10	4.50 ± 0.89**
E January 1978	11.60 ± 2.79	-	-	19.37 ± 1.60	6.98 ± 3.34

* Data taken from Rastogi et al.[2]; frogs were kept at 28 °C, instead of 24 °C. ** Significance of difference vs control $p < 0.01$.

importance of light and temperature in the regulation of the annual testicular cycle in this species is well established, little is known about their effects on androgen production[2,4,5]. In this study the effects of light and temperature on the production of androgens were examined in *Rana esculenta* at different stages of testicular activity.

Materials and methods. Sexually mature specimens of *Rana esculenta* were collected in the months of January, June, July and October 1977 and January 1978. Photothermal treatments (table) were continued for a total of 7 days. Each group consisted of 8–10 frogs plus controls. Each frog was anaesthesized with MS 222 and the blood was collected in heparinized micro-tubes directly from the conus arteriosus. For each determination 200 µl of plasma was used. ^{3}H-testosterone, sp. act. 100 Ci/mM was used. Testosterone-3-oxime BSA was employed as antiserum. It reacts appreciably with dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) and measurements are therefore referred to as total androgens. Results were analyzed for significance using Student's t-test.

Results and discussion. The table summarizes the changes in average plasma androgen levels at different temperatures during different periods of the year. In winter (experiments A and E) plasma androgens increased significantly at high temperature only in frogs provided with a light source. In animals kept at 24 °C but in total darkness, and those kept at 4 °C and 12:12 h light-dark cycle, the plasma androgen levels remained unaltered. This confirms our previous observations concerning the importance of light[2]. In summer frogs (experiments B and C), on the other hand, none of the combinations of temperature and light used in this study had any effect. In October (experiment D), however, low temperature stimulated the plasma androgens, while high temperature had no significant effect.

The discussion would be quite simple if we had information on the circulating levels of gonadotropins during the year. Since this is totally missing from the literature the task of discussing results like the present one is quite arduous. The high temperature-induced increase of plasma androgens in winter frogs could be explained on the basis of our knowledge that high temperatures stimulate pituitary gonadotropin secretion[2,5,6]. October frogs show an increase in plasma androgens at low temperature, and observations in nature confirm that during this period of the year the falling environmental temperature favours a rise in plasma androgens. In summer frogs, the low androgen levels remain unaltered under any experimentally-produced environmental condition. Thus, although this is fraught with risk, we venture to speculate that there probably exists an endogenous rhythm for the sensitivity of testicular steroidogenic sites to the pituitary gonadotropins, which in turn are under the control of environmental variables. In addition, the present data demonstrate that the effects of environmental stimuli on the reproductive biology of *Rana esculenta* are considerably more complex than suggested earlier[2,5,6]. In fact it is shown that different parameters, like pituitary gonadotropic activity, the response of the testis to pituitary gonadotropins, and the response of peripheral androgen target organs, are not equally temperature-sensitive. Thus follow-up research is certainly needed.

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Changes in adenylate cyclase and 5′-nucleotidase activities in liver membranes from alloxan diabetic rats

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Summary. Liver membrane adenylate cyclase activity was significantly higher and 5′-nucleotidase activity significantly lower in alloxan diabetic rats compared with normal rats.

The enzyme 5′-nucleotidase (EC3.1.3.31) is located in the external part of the plasma membrane. Adenylate cyclase (EC4.6.1.1.) is situated in the interior part of the plasma membrane. The hepatic level of adenosine 3′5′-monophosphate (cyclic AMP, cAMP), the product of adenylate cyclase, is increased in alloxan diabetic rats[1]. Adenosine, the product of 5′-nucleotidase, is rapidly translocated from the outside into cells where it is metabolized[2]. This causes steady state levels to be very low. In previous studies adenosine has been linked with insulin action[3] and has also been found to have a strong inhibitory influence on adenylate cyclase activity in liver membranes[4]. Recently, recipro-

cal alterations in 5′-nucleotidase and adenylate cyclase activities were found during cartilage maturation[5]. The present study concerns the effect of alloxan-induced insulinopenia in rats on the activities of hepatic 5′-nucleotidase and adenylate cyclase. The study was also performed on rats investigated concerning the effect of zinc on wound healing. The results of this latter investigation are to be presented elsewhere.

Materials and methods. 35 male Sprague-Dawley rats weighing 180–230 g were used. They received standard rat chow (Astra-Ewos, Sweden) and had free access to drinking water. 17 animals were made diabetic by rapid i.v. injection of alloxan monohydrate (40 mg/kg) in 0.9% saline. Blood glucose above 7.8 mmoles/l combined with glucosuria (Clinistix® positive) indicated diabetes. Because of the simultaneous wound healing study all the animals, diabetics and controls, were operated with a piece of sponge placed s.c. 14 days after alloxan treatment. Directly thereafter 10 rats were injected with 75 µg ZnAc in a single i.m. dose while 8 rats were injected with the same dose for 4 days. All animals were sacrificed on the same day. The rats were killed with ether. The abdominal and chest cavities were opened and 5 ml of blood was drawn from the right heart chamber whereupon 50 ml of 0.9% saline was injected into the left heart chamber. The liver was removed and about 1 cm^3 was taken for enzyme studies.

The method for preparation of a crude membrane fraction was the same as that presented elsewhere[4] except that the liver homogenates were frozen in liquid nitrogen before further preparation. The incubation medium contained 50 mmoles/l Tris-HCl, pH 7.6, 1 mmole/l 1-methyl, 3-isobutyl-xanthine, 4 mmoles/l $MnCl_2$, 4 mmoles/l ATP and 0.1 mmoles/l GMP-PNP. To 1.25 ml of medium was added glucagen (final concentration 56 nmoles/l). Incubation was performed at 37 °C in a shaking water bath and the reaction was started by the addition of 50 µl of liver membrane preparation giving a final protein concentration of 39–132 µg/ml. Total volume was 1.32 ml. At 10 min a 1-ml sample was taken and heated at 100 °C for 4 min. Zero time samples were obtained by adding membrane preparation to medium at 100 °C. Cyclic AMP was determined according to a method presented elsewhere[4]. Protein and glucose were measured by the techniques of Hartree and Marks, respectively[6,7]. 5′-nucleotidase was measured spectrophotometrically according to Belfield and Goldberg[8].

Results. The activity of 5′-nucleotidase in diabetic rats was significantly lower, 50.3 ± 17.8 milliunits/mg protein, than in controls, 65.7 ± 10.2 milliunits/mg protein ($p < 0.01$). The reverse was true for the adenylate cyclase activity with 3.14 ± 0.57 and 2.76 ± 0.42 nmoles cAMP (mg protein)$^{-1}$ (10 min)$^{-1}$ for the diabetics and controls, respectively ($p < 0.05$) (table). The addition of alloxan (1 mmole/l) to the incubation media was found not to affect enzyme activities. Taking all observations from diabetic and control animals together there was a significant inverse correlation between 5′-nucleotidase and adenylate cyclase activities ($r = -0.46$, $p < 0.01$) (figure). Zinc treatment did not have any influence on zinc concentration in hepatic tissue or on the 2 enzyme activities studied.

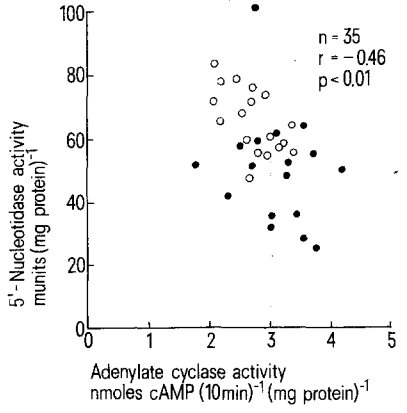
Discussion. Adenosine, the product of 5′-nucleotidase, is a potent inhibitor of adenylate cyclase activity in the liver[4]. The pronounced decrease in 5′-nucleotidase activity and the increase in adenylate cyclase activity in the diabetic rats may be interrelated through adenosine levels. Reciprocal alterations in these enzyme activities were recently found also in cartilage during maturation[5], and may have been the effect of insulin-like factors of the somatomedin group. Bhathena et al. recently found liver 5′-nucleotidase activity to be considerably lower in diabetic rats compared with controls[9]. This connection between the activity of 5′-nucleotidase and the diabetic state of streptozotocin treated rats was also recognized by Soman and Felig[10], who furthermore found the enzyme activity to be influenced by insulin supplementation. They also proposed that the increased glucagon stimulable adenylate cyclase activity of diabetic animals might be an effect of increased glucagon binding, which is in conflict with the results of others[9,11] on down-regulation of glucagon receptors in the hyperglucagonemia of insulin deficient states.

In conclusion, alloxan diabetes is associated with a decrease in hepatic 5′-nucleotidase activity and an inversely proportional increase in adenylate cyclase activity. These changes may possibly be interrelated through adenosine levels. It remains to be elucidated whether these enzyme perturbations are related to insulin deficiency or to some other aspect of the diabetic state.

Hepatic membrane enzyme activities in diabetic and control animals

	Diabetics	Controls	p
Adenylate cyclase activity [nmoles cAMP (10 min)$^{-1}$ (mg protein)$^{-1}$]	3.14± 0.57	2.76± 0.42	<0.05
5′-nucleotidase activity (munils/mg protein)	50.3 ±17.8	65.7 ±10.2	<0.01
n	17	18	

Mean ± SD.



Relationship between hepatic 5′-nucleotidase and adenylate cyclase activities in diabetic (●) and control (○) animals.

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