tissue used in each extraction varied from 0.5 g to 1.0 g. The qualitative and quantitative estimation of tryptophan was made by 2-dimensional chromatography as described by Plummer[11]. The activity of IAA-oxidase was determined on the same day as described[12] with slight modification. The data are the averages of 3 determinations from each combined sample of the plant tissue.

Data presented in figure 1 reveal that the average root length decreased with increasing concentrations of dikegulac-sodium. However, the decrease in weight of roots was less pronounced than the decrease in length, as the roots were relatively thicker and stunted for the dikegulac-sodium-treated seedlings. At higher concentrations (250–750 mg/l) the roots turned brown 4 days after radicle emergence. The radicles of treated seedlings became negatively geotropic while control and decapped roots remained normal. Lower concentrations (50–100 mg/l) did not show any effect on geotropic response. The highest content of tryptophan was in untreated roots: its content decreased with increasing amounts of dikegulac-sodium (figure 2) and was at its lowest in the decapped roots. The activity of IAA-oxidase was higher in all dikegulac-sodium-treated roots than in controls (table).

The action of dikegulac-sodium is similar to that of morphactins[13]. Chifford[14] indicated that the inhibitory effects of morphactins on root growth (dikegulac-sodium shows similar effects with respect to root growth and geotropic response) would not fit the assumption of the Cholodny-Went theory[2] that endogenous auxin in the root is supraoptimal for growth. The present study confirms Chifford's view. It reveals that a reduction in tryptophan and IAA level tends to give rise to a sub-optimal level of IAA and is directly proportional to root growth and inhibition of negative geotropic response. The content of tryptophan and IAA in the untreated roots may reflect the accumulation in the root tips, derived from the acropetal stream. It may therefore be concluded that dikegulac-sodium interferes with geotropic response and thus acts as a endogenous growth regulator and can modify tryptophan level and IAA-oxidase activity in roots.

Effect of dikegulac-sodium on IAA-oxidase activity of *Glycine max* roots after 7th day of seeding

| Dikegulac-sodium (mg/l) | mg/IAA destroyed/g/h |
|---|---|
| Distilled water | 0.01 ±0.002 |
| 50 | 0.036±0.001 |
| 100 | 0.053±0.003 |
| 250 | 0.061±0.002 |
| 500 | 0.082±0.004 |
| 750 | 0.094±0.001 |

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2 N. Cholodny, Jb. Wiss. Bot. *65*, 477 (1928).
3 S.S. Purohit, Thesis, University of Udaipur, India, 1977.
4 S.S. Purohit, Comp. Physiol. Ecol. *4*, 264 (1979).
5 S.S. Purohit, Comp. Physiol. Ecol. *5*, 24 (1980).
6 S.S. Purohit, Comp. Physiol. Ecol. *5*, 83 (1980).
7 S.S. Purohit, Curr. Sci. *49*, 403 (1980).
8 S.S. Purohit, Comp. Physiol. Ecol., in press (1981).
9 S.S. Purohit and K. Chandra, Phyton, Argentina, *39*, in press (1981).
10 S.S. Purohit and K. Chandra, Curr. Sci. *49*, 635 (1980).
11 D.T. Plummer, An Introduction to Practical Biochemistry, 2nd edn. McGraw-Hill Company, London 1978.
12 O.P. Srivastava and R.B. VanHaystee, Can. J. Bot. *51*, 2207 (1973).
13 P.F. Bocion and W.H. de Silva, in: Plant Growth Regulation, 9th International Conference on Plant Growth Substances, p. 189. Ed. P.E. Pilet. Springer Verlag, Berlin 1977.
14 P.E. Chifford, Z. Pflanzenphysiol. *90*, 309 (1978).

## Influence of photoperiodism on high temperature-induced testicular recrudescence in the green frog[1]

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*Summary.* In connection with the circannual testicular rhythm *Rana esculenta* does not seem to be a strictly photoperiodic species. In fact testicular growth can be induced (at a favourable temperature) with a daily 2-h light pulse as well as with a daily 12-h light pulse.

We have evidence that the testicular cycle in *Rana esculenta* is regulated by an endogenous circannual rhythm. Both light and temperature have remarkable influence on the annual testicular cycle in this species. Light, however, has been considered to have only a permissive role in facilitating the temperature response which apparently is the more direct modifier of testicular recrudescence and spermatogenic arrest. At favourable temperatures (15–24 °C) a positive testicular response to temperature is obtained if the animals are maintained under a 12:12 LD cycle. Darkness has deleterious effects[2–4].

Is *R. esculenta* a photoperiodic species? We have no answer to this question so far. This preliminary investigation was carried out to study the effects of a 2-h light pulse on the testicular growth in frogs having involuted and quiescent tests and maintained at a constant temperature of 20 °C.

Animals procured in January and February were acclimatized for 1 week in the laboratory, before being transferred to the photo-thermo-static chambers. Each group was composed of 20 frogs. The 4 groups of experimental animals for each month received a daily 2-h light pulse respectively at 09.00, 11.00, 13.00 and 15.00 h. 10 animals each were sacrificed respectively at 15 and 30 days after the beginning of the experiment. 2 additional groups were maintained under total darkness or with a 12:12 LD cycle (the 12-h light pulse beginning at 09.00 h) and a constant temperature of 20 °C for 30 days. Initial values were obtained by sacrificing a batch of 8 frogs at the start of each experiment. Animals were weighed, and then sacrificed by decapitation. The testes were taken out, weighed, and fixed in Bouin's fluid for histological examination.

Results are summarized in the table. In January a daily 2-h light pulse, given at any hour of the day (from 09.00 h–15.00 h), did not favour testicular growth at high temperature. The same holds true for animals maintained in total darkness. On the contrary, a daily 12-h light pulse induced

Influence of photoperiod on testicular recrudescence in high-temperature-maintained winter frogs

| Treatments | Gonosomatic index | | Relative number of I SPC cysts/ seminiferous tubule | |
|---|---|---|---|---|
| | January | February | January | February |
| Initial control | 0.21±0.02 | 0.21±0.02 | 0.83±0.16 | 0.92±0.09 |
| Dark | 0.24±0.02 | 0.26±0.02[c] | 0.98±0.10 | 1.08±0.13 |
| LD 12:12 | 0.36±0.03[a,d] | 0.35±0.02[a,d] | 3.84±0.46[a,d] | 3.56±0.59[a,d] |
| 2-h light pulse | | | | |
| 09.00–11.00 h | 0.19±0.02 | 0.33±0.03[a,d] | 0.76±0.08 | 2.93±0.37[a,d] |
| 11.00–13.00 h | 0.25±0.04 | 0.30±0.02[a] | 0.82±0.09 | 2.99±0.42[a,d] |
| 13.00–15.00 h | 0.25±0.04 | 0.28±0.02[a] | 0.91±0.10 | 2.85±0.26[a,d] |
| 15.00–17.00 h | 0.23±0.04 | 0.27±0.02[b] | 0.86±0.03 | 2.87±0.63[a,d] |

[a] Significance of difference vs initial control ($p<0.01$); [b] significance of difference vs initial control ($p<0.02$); [c] significance of difference vs initial control ($p<0.05$); [d] significance of difference vs dark-treated frogs ($p<0.01$).

a significant increase in testis weight ($p<0.01$). In February the initial controls did not show any change in the testis as compared with January controls. The 2-h light pulse, as well as the LD 12:12 cycle however, induced a highly significant testicular growth. In comparison, although it was significantly higher than in the initial controls ($p<0.05$) the testis weight in dark-treated frogs was significantly lower than in the LD 12:12 and LD 2:22 (09.00–11.00 pulse) frogs ($p<0.01$).

In February, furthermore, except for frogs having a daily 2-h light pulse beginning at 09.00 h, the weight of LD 12:12 frogs' testes was far higher than in those given the short light pulse for 30 days beginning respectively at 11.00, 13.00 and 15.00 h.

The testis in January and February frogs usually contains numerous sperm bundles attached to Sertoli cells, some secondary spermatogonia cysts and a few spermatocytes. Histological examination of the testis in all experimental groups revealed evident signs of spermiation (presence of free sperm masses in the lumen of seminiferous tubules and efferent ductules).

Spermatogonial multiplication was enhanced in all groups. The differences were noted at the level of germinal cysts containing primary spermatocytes. Few spermatocyte cysts were found in dark-treated frogs or in those given the 2-h light pulse in January. In February the testis of all the 4 2-h light pulse groups contained numerous cysts containing primary spermatocytes. The number of such cysts was still greater in January and February frogs treated with 12:12 LD cycle (table).

These results indicate that in January the 2-h light pulse is not sufficient to induce testicular growth. On the contrary, in February, the 2-h light pulse, given at any time of the day, favoured the temperature-induced testicular growth, as in the LD12:12 frogs. In addition to this, the February dark-treated frogs did not show an increase in the number of primary spermatocytes.

It should be noted that in nature winter stasis terminates and testicular recrudescence sets in by the end of February or the beginning of March, when both day length and environmental temperature start increasing.

Although the importance of light in the temperature response of the testis has once again been shown, it is also evident that *R. esculenta* is not a strictly photoperiodic species. In fact, scanning the major part of the day at 2-h intervals showed that the photo-inducible phase, of the testicular rhythm during recrudescence (if it exists in *R. esculenta*), falls between 09.00 h and 17.00 h. But in this span of 8 h even a 2-h light pulse produced significant testicular growth with a probable peak at 09.00 h. A longer light pulse (12:12 LD), however, was still more stimulating which may mean that a longer light regimen might have an accumulative effect on testicular rhythm. In some teleosts it has been demonstrated that a circadian mechanism is involved for photoperiodic measurement[5,6]. Further experiments will have to be conducted to test whether such a mechanism also exists in the frog.

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2 R.K. Rastogi, Boll. Zool. *43*, 151 (1976).
3 R.K. Rastogi, L. Iela, G. Delrio, M. Di Meglio, A. Russo and G. Chieffi, J. exp. Zool. *206*, 49 (1978).
4 R.K. Rastogi, Gen. comp. Endocr. *40*, 309 (1980).
5 B.I. Sundararaj and S. Vasal, J. Fish. Res. Board Can. *33*, 959 (1976).
6 B. Baggerman, Gen. comp. Endocr. suppl. *3*, 466 (1972).

## Reflex motility of the stomach evoked by electrical stimulation of the hepatic vagus nerve

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*Summary.* Electrical stimulation of the cranial end of the cut hepatic branch of the vagus nerve produced an increase in the motility of the empty stomach in the rat, whereas systemic injection of insulin produced a gradual increase in the motility.

It has been recognized that insulin and glucose alter gastric motility[2,3] and act by stimulating the neural glucose-sensitive mechanism located within the brain[4–6]. However, behavioural and neurophysiological studies have pointed out the existence of a neural mechanism responsive to glucose in the hepatic portal vessels[7–9]. Previously we noted that portal injection of glucose specifically affected the efferent activity of the vagus nerve innervating the stomach, and presumed that there might be a neural system modulating gastric function through hepatic vagal afferents and gastric