cohol intoxication, the disruption of intracellul0ar autoregulation.

A comparison of the experimental results with clinical data and data contained in the literature permits us to conclude that the hematological state in the case of short-term alcohol intoxication under ecologically unfavorable conditions tends to resemble that in the case of long-term excessive alcohol intoxication. The resultant changes in the erythrocyte system can undoubtedly be regarded as one of the contributing factors in ecological-narcological diseases.

REFERENCES

1. V. K. Al'pidovskii, *Gematol. Transfuziol.*, № 9, 45-50 (1983).
2. V. V. Bitenskii and R. Ya. Mar'yanchik, in: *Urgent Problems of Clinical and Social Rehabilitation of Alcoholics* [in Russian], Moscow (1979), pp. 43-45.
3. G. N. Degteva, P. I. Sidorov, A. G. Marachev, *et al., Zh. Nevropat. Psikhiatr.*, № 2, 230-235 (1987).
4. N. G. Demeshkevich and L. A. Serebryanyi, *Fel'd. Akusher.*, № 7, 19-22 (1979).
5. I. A. Kassirskii and G. A. Alekseev, *Clinical Hematology* [in Russian], Moscow (1970).
6. Yu. P. Lisitsyn and P. I. Sidorov, *Alcoholism* [in Russian], Moscow (1990).
7. E. N. Mosyagina, *Erythrocyte Equilibrium in Health and Pathology* [in Russian], Moscow (1962).
8. L. F. Panchenko, V. G. Antonenkov, and V. A. Yakovchenko, *Ethanol and Metabolism* [in Russian], Minsk (1982), pp. 135-147.
9. P. I. Sidorov, A. G. Solov'ev, and Z. I. Taraskina, in: *Ways of Improving the Efficiency of the Wood-Working Industry* [in Russian], Arkhangelsk (1989), pp. 171-172.
10. A. G. Solov'ev and Z. I. Taraskina, in: *Ecological and Physiological Aspects of Adaptation* [in Russian], Moscow (1988), pp. 217-218.
11. V. N. Chernigovskii (Ed.), *Physiology of the Blood System: Physiology of Erythropoiesis* [in Russian], Leningrad (1979).
12. Yu. L. Shapiro, V. A. Sycheva, and A. T. Stepanovskaya, in: *Proc. Kuibyshev Regional Conference of Medical-Prophylactic Establishments*, Moscow (1971), pp. 345-348.
13. E. Eschwegee *et al., J. Clin. Path.*, **31**, 654-658 (1978).
14. F. Wisloff and D. Boman, *Acta Med. Scand.*, **205**, 237-242 (1978).

Chronobiological Regularities of Amphibian Metamorphosis

Yu. A. Romanov, M. A. Badretdinov, and I. F. Golichenkova

UDC 612.018:[577.175.444+577.175.328].019.08

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 8, pp. 197-199, August, 1993
Original article submitted February 19, 1993

Key Words: *amphibians; metamorphosis; thyroxine; prolactin; chronobiology*

The metamorphosis of amphibians, one of the most important biological processes, has been fairly thoroughly studied. The main controlling mechanisms of this process have also been described [1,4-6,10]. However, there is little information on the chronobiological regularities of metamorphosis and on the relationship between the biological action of such metamorphogenic hormones as thyroxine and prolactin and the photoperiodic conditions [3,7,9,11].

Biology Department, Medico-Biological Faculty, Russian State Medical University, Moscow

The purpose of this work was to make a chronobiological study of the effect of thyroxine and prolactin on certain temporal characteristics of metamorphosis of tailless amphibians in relation to the phase of the light-dark cycle.

MATERIALS AND METHODS

In our experiments we used *Rana temporaria* larvae in the state of prometamorphosis, and also in the state of metamorphosis proper from the 23rd to the 29th stage of development [8]. The light-

0007-4888/93/0008-1005$12.50

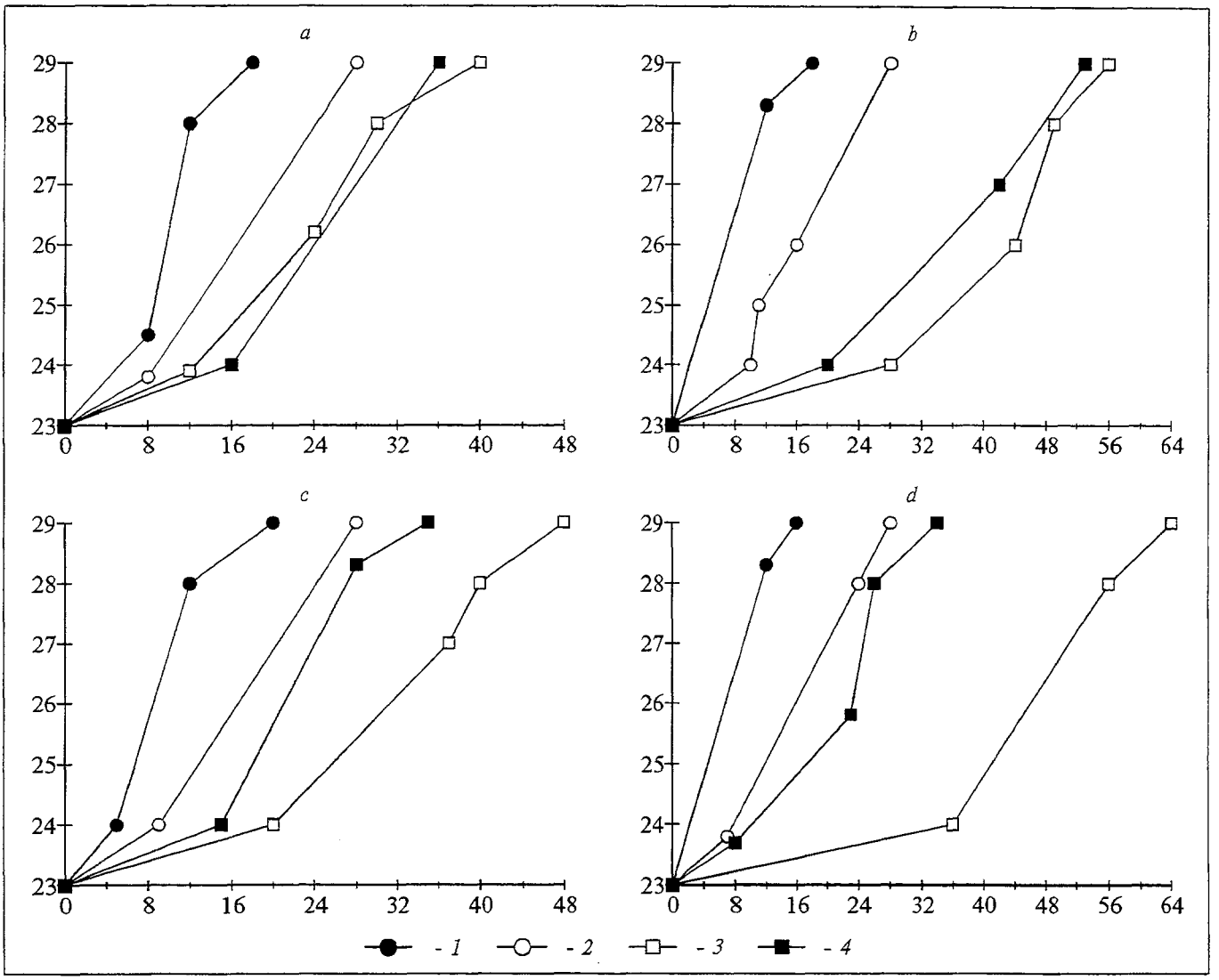

Fig. 1. Changes in τ_{av} for larvae in experimental series I–IV. Abscissa: time, days; ordinate: stage of development. *a*) group 1; *b*) group 2; *c*) group 3; *d*) group 4; T: thyroxine; P: prolactin; C_{pure}: control (tap water); C_a: alkaline water.

dark cycle (L:D) was 14-10 hours (the light phase from 8:00 to 22:00 h), and the intensity of illumination was 350-400 lux. Series I of the experiments consisted of the control experiments (the animals were constantly kept in dechlorinated tap water). In series II, III, and IV the larvae were kept for 6 hours at different times during a 24-hour period in water containing alkali (alkaline control experiment), thyroxine, and prolactin, respectively. In each of the series of experiments the larvae were divided into four group. Group 1 larvae were exposed to the above-mentioned substances from 6:00 to 12:00 h (transition from D to L); group 2 larvae from 12:00 to 18:00 h (L); group 3 larvae from 18:00 to 24:00 h (transition from L to D); and group 4 larvae from 24:00 to 6:00 h (D). The concentration of alkali (NaOH) in the medium was 2×10^{-6} g/liter, and of the hormones 2×10^{-7} g/liter. We used bull prolactin (obtained from the Research Institute of Endocrinology and Hormone Chemistry, Russian Academy of Medical Sciences) and L-thyroxine (Reanal, Hungary). For elimination of the group effect the animals were kept in crystallizing pans with 2 liters of water per 35-40 larvae, and the water was changed every 18 hours [2]. To describe the metamorphosis quantitatively we determined, first, the average time it took the larvae to pass through each stage of development from the start of the experiment together with the preceding stage (τ_{av}), using the formula of the arithmetic weighted mean for a series; and, second, the average time it took the larvae to pass through the separate stages of development (τ_{av}) by subtracting τ_{av} of the preceding stage from t_{av} of the given stage. The speed (or rate) at which the larvae passed through the separate stages of development was assessed from plots of the completion parameters as a function of

time; the completion parameters corresponded to a certain number of tadpoles at given stage of development for each day of the experiment. All the quantitative data were processed biometrically; the differences were considered to be reliable for $p \leq 0.05$.

RESULTS

As can be seen from Fig. 1, the larvae in series I of experiments developed from the 23rd to the 29th stage in 28 days on the average.

The alkaline water prolonged the metamorphosis. But its effect varied, depending on the time the larvae were held in the water (50 days for group 3 larvae, 33 days for group 4 larvae).

Thyroxine, as might have been expected, accelerated metamorphosis in all the time groups. However, there are certain differences in the metamorphogenic influence of the hormone, depending on the phase of the light-dark cycle. When thyroxine was used in periods of transition from the dark to the light phase and vice versa (group 1 and group 3, respectively), the period of larvae development was reduced by 70 and 68%, respectively, as compared with the alkaline control experiment. When thyroxine was used in the light and dark periods, development was shortened by only 55 and 52%, respectively.

Of particular interest are the results of series IV of the experiments, in which prolactin was used. The effect of this hormone on the larvae of group 2 and 3 practically did not change the time in which the metamorphosis process was completed. The use of prolactin in the dark period greatly slowed down development (by 82%). But if prolactin was used from 6:00 to 12:00 h (transition from D to L, group 1), the development of the larvae accelerated by 24% as compared with the alkaline control experiment. Thus, it was shown that the biological effect of prolactin can vary drastically, depending on the particular period of the 24-hour cycle during which this hormone is used. It is interesting to note that there were intrastage differences in the shensitivity to the hormone, depending on the light-dark phase. Thus, the acceleration of larvae development by thyroxine was probably due to a reduction of the duration of the stages in which development normally slows down (23rd to 25th stage).

In the case of tadpoles stimulated by prolactin (group 1) development was accelerated in the first half or in the middle of the stages. In group 4 larvae, which underwent a considerably slowdown in their development, tha rate of passage through the stages was reduced, mainly in the first half. Thus, a chronobiological approach to a study of metamorphosis clearly shows the hormonal effect on each stage of development.

Hence, the findings show that there are obvious differences between the metamorphogenic action of thyroxine and prolactin, depending on the phase of the light-dark cycle in which they were used. According to generally accepted notions on the hormonal regulation of metamorphosis [4,5], it is these hormones, as well as hypothalamic neurosecretion, that play a leading role in controlling the metamorphosis process. Prometamorphosis is characterized by a high level of prolactin and a low level of thyroxine. With the onset of metamorphosis these correlations change, apparently due to the establishment of positive feedback between the thyroid hormone and the hypothalamus. The data presented above show that it is necessary to define more precisely the principles of hormonal interaction in the regulation of amphibian metamorphosis, taking into account the chronophysiological effect of prolactin and thyroxine.

We believe that it would be particularly interesting to compare the results we obtained with the data yielded by the use of a "fretted" light regime [12]. In the work cited the hormonal influence on metamorphosis is shown to be due to a change in the hormonal state of the body in response to the light-dark effect. It is possible that, by using the method of such detailed, stage-by-stage "scanning" of the body under conditions of a controlled photoperiod, one could get a sufficiently clear picture of the hormonal control of metamorphosis by exogenously administered hormones.

REFERENCES

1. M. S. Mitskevich, *Hormonal Regulations in the Ontogenesis of Animals* [in Russian], Moscow (1978).
2. S. S. Shvarts, O. A. Pestova, L. A. Dobrinskaya, and G. G. Runkova, *Effect of the Group in Populations of Aquatic Animals* [in Russian], Moscow (1976).
3. C. B. Breax and A. H. Merier, *Amer. Midland Natur.*, **85**, № 1, 267-271 (1971).
4. W. Etkin, in: *Hormones and the Environment*, Cambrige (1970), pp. 137-153.
5. W. Etkin, in: *Pioneers in Neuroendocrinology*, New York (1978), pp. 157-174.
6. H. Fox *Amphibian Morphogenesis*, Clifton, New Jersey (1984).
7. M. M. Joseph, in: *Chronobiology*, Stuttgart-Tokyo (1974), pp. 142-146.
8. F. Kopsch, *Die Entwiklung des Braunen Grasfrosches Rana fusca Roesel*, Stuttgart (1952).
9. P. Licht. D. C. Cohen, and H. A. Bern, *Gen. Comp. Endocr.*, **18**, № 2, 391-394 (1972).
10. R. Wasserburg, *J. Theor. Biol.*, **118**, 171-181 (1986).
11. M. L. Wright, E. K. Frim, V. A. Bonak, and C. Baril, *Gen. Comp. Endocr.*, **63**, 51-61 (1986).
12. M. L. Wright, S. L. Jorey, Y. M. Myers, *et al.*, *Gen. Growth Differ.*, **30**, 315-323 (1988).