HERPETOLOGICAL JOURNAL, Vol. 2, pp. 125–132 (1992)

EFFECTS OF LOW TEMPERATURE ON TESTICULAR CELLS IN THE MARBLED NEWT, *TRITURUS MARMORATUS* (CAUDATA, SALAMANDRIDAE)

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(Accepted 17.6.91)

ABSTRACT

The response of the different germ cell types and glandular tissue of the testis to low temperatures (4°C) and long photoperiods (16L:8D) was studied in the marbled newt (*Triturus marmoratus*) by histologic quantitative methods in the three periods of the annual cycle: quiescence (January-March), germ cell proliferation up to round spermatids (April-June), and spermiogenesis (July-September). Together with each group of cold-exposed newts, another group was maintained at mild temperature (20°C) over the same long photoperiod. At the beginning and end of each period, initial and final wild controls were collected. In the quiescent period, only spermatogonial proliferation was observed in the initial and final controls as well as in the cold-exposed newts. The newts kept at 20°C developed spermatogenesis up to the round spermatid level. At the end of the germ cell proliferation period, the final controls showed round spermatids; the newts exposed to 20°C developed complete spermatogenesis; and the newts maintained at 4°C only presented spermatogonial proliferation. At the end of the spermiogenesis period, the final controls and the newts kept at 20°C showed complete spermatogenesis and developed glandular tissue whereas the newts exposed to 4°C only had round spermatids and had no glandular tissue. Present results suggest that although low temperature does not affect spermatogonium proliferation it impedes both the subsequent steps in spermatogenesis and the development of glandular tissue.

INTRODUCTION

Photoperiod and temperature are the most important external factors controlling the reproductive cycle in amphibians (Galgano & Flachetti, 1940; Cei, 1944; Lofts, 1974). In urodele amphibians inhabiting cold-temperate areas spermatogenesis occurs during spring and summer when the photoperiod is longer and temperature is higher, whereas in autumn and winter, when the photoperiod is short and temperature is low, the testis remains quiescent (Lofts, 1974; Sáez, Fraile & Paniagua, 1990). The duration of the spermatogenesis period varies for the same species depending on the latitude and altitude of the geographic area (van Oordt, 1956; Rouy, 1972). Experiments in several urodele species have shown that 12-16 hr of light per day and temperature of 20°C induce spermatogenesis even during the period of testicular quiescence (Werner, 1969; Steinborn, 1984; Fraile, Paniagua & Rodríguez, 1988). Werner (1969) in *Plethodon cinereus* and Steinborn (1984) in *Triturus cristatus* found that mild temperatures (20-22°C) induce development from spermatogonia into spermatocytes in the newts exposed to short photoperiods (less than 8 hr of light daily). Similar findings were reported by Fraile *et al.* (1988) in marbled newts (*Triturus marmoratus*) kept in complete darkness. However, longer photoperiods (12-16 hr of light) are necessary for obtaining complete spermatogenesis (Werner, 1969; Fraile, Paniagua, Rodríguez & Sáez, 1989a). The effects of moderately low temperatures (10-11°C) have been studied by Werner (1969) and Steinborn (1984). Both authors observed that these temperatures induce spermatocyte formation if the photoperiod is long (12-16 hr), although higher temperatures are required to achieve complete spermatogenesis. These experiments suggest that: (1) neither photoperiod nor temperature controls the initial phase of spermatogenic development; and (2) both long photoperiods and mild temperatures are necessary for meiosis and spermiogenesis.

Nevertheless, these results differ from the data reported by Ifft (1942) who failed to observe development from spermatogonia into spermatocytes in the urodele *Notophthalmus viridescens* maintained at 8°C even when the animals were exposed to long photoperiods. In addition, the experiments on temperature in anurans indicate that mild temperatures are a prerequisite for spermatocyte development (Lofts, 1974; Rastogi, Iela, Sasena & Chiefi, 1976).

These dissimilarities in results might be attributed to differences between species, but also to the period of the cycle in which the animals were exposed, the duration of the exposure and the exact temperature of exposure.

The present study concerns the influence of a temperature of 4°C on each germ cell type and glandular tissue in the testis of the marbled newt in the three different periods of the annual testicular cycle. The temperature was chosen because it was the average temperature during December-January in the area inhabited by the newts. The newts were exposed to the optimal photoperiod according to previous studies (Fraile *et al.*, 1988, 1989a; Fraile, Paniagua, Rodríguez & Sáez, 1989b) and the results were compared with those obtained in newts exposed to the same photoperiod and mild temperature as well as with those obtained in control marbled newts exposed to the environmental temperature and the natural photoperiod in the wild.

MATERIALS AND METHODS

Twenty-four marbled newts (*Triturus marmoratus marmoratus* Latreille) were collected from forested areas in the Province of León (Spain) on December 30th (quiescent period), March 30th (end of the quiescent period), and June 30th (end of the period of germ cell proliferation and meiosis and beginning of the spermiogenesis period) (Sáez *et al.*, 1990). In order to eliminate the influence of body weight in the experimental results, only newts weighing between 9.0 and 9.5 g were selected. The 24 newts captured on each of these days were sorted into three groups of eight animals. One group was killed the next day and used as initial or final controls for the three successive experiments. The other two groups were maintained in the laboratory for three months. Each group was kept in a glass

aquarium (1 x 1 x 0.4 m) containing fresh water up to a depth of 20 cm. A solid surface (a flat-based roughly pyramid-shaped rock) was provided so that the newts could either swim or rest on this surface. Lighting was supplied by cool, white, 14 W fluorescent lamps with a wavelength distribution from 350 to 710 nm. Lighting conditions were maintained at a 16L:8D photoperiod by automatic timers. Each group was kept in a different room provided with a thermostat. Water and air temperatures were maintained at 20±1°C for one group, and at 4±1°C for the other group. The animals received food every two days. To obtain final controls at the end of the spermatogenesis period, eight newts were collected on September 30th and killed the day after.

The animals were weighed, anaesthetized with methanesulphonate, and fixed by perfusion through the aortic cone with the Karnovsky fixative (3% phosphate-buffered glutaraldehyde-paraformaldehyde, mixed in equal proportions) for 30 min. Following this, both testes were removed and weighed. Testicular volumes were calculated by water displacement. The right testes were sliced into small fragments which were embedded in epoxy resin. Semi-thin sections were stained with toluidine blue. The left testes were fixed for an additional 6 hr in the same fixative, dehydrated, and embedded in paraffin. The blocks were used for quantitative studies. Since the wave of germ cell differentiation in the newt testis progresses from the posterior to the anterior pole, only sagittal sections of the whole testis were suitable for quantitative studies. For this purpose, five 6µm-thick sagittal sections of each left testis at points 1/6, 1/3, 1/2, 2/3 and 5/6 of the transverse testicular diameter were selected and stained with haematoxylin and eosin. In each testis, the areas occupied in the five sections by each germ cell type (including their accompanying Sertoli cells and connective tissue cells) and the glandular tissue (developed Leydig cells) were measured with a semi-automatic image analyzer (Kontron, Zeiss, Oberkochen, FRG). The resulting values were divided by the total surface area of the five sections thus obtaining the volume densities of each germ cell type. The absolute volumes per testis for each cell type were obtained by multiplying volume densities by testicular volume and by a correction factor (0.76) which is the result of the transformation of testicular volume after embedding. This factor was previously determined from 50 newt testes by water displacement.

Means and SD for each group of newts were calculated from the values obtained for each animal. Comparison of the means between the different groups in each experiment was carried out by a one-way ANOVA test. For the parameters showing significant differences, comparison between each pair of means was carried out by the two-sample *t*-test.

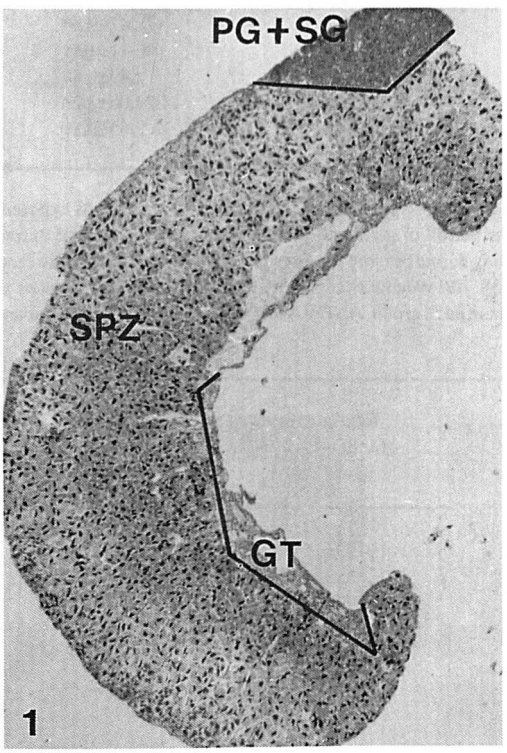

Fig. 1. Testicular lobe from a marbled newt exposed to 40°C and 16L:8D for three months during the period of testicular quiescence. The distribution of germ cell zones is indicated using black lines. PG: primary spermatogonia; SG: secondary spermatogonia; SPZ: spermatozoon bundles; GT: glandular tissue. Haematoxylin and eosin. x15.

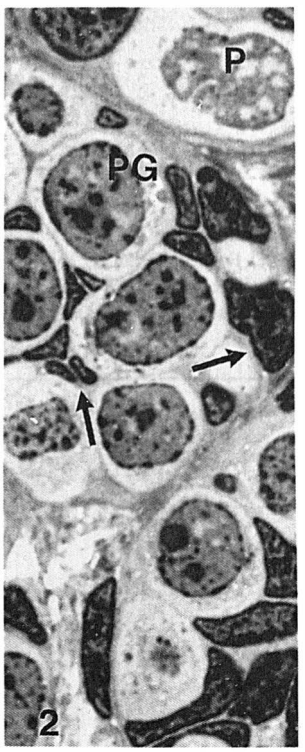

Fig. 2. Primordial germ cells (P) and primary spermatogonia (PG) in the contralateral testis from the same newt. Each primary spermatogonia is completely surrounded by follicular cells (arrows). Toluidine blue. x940.

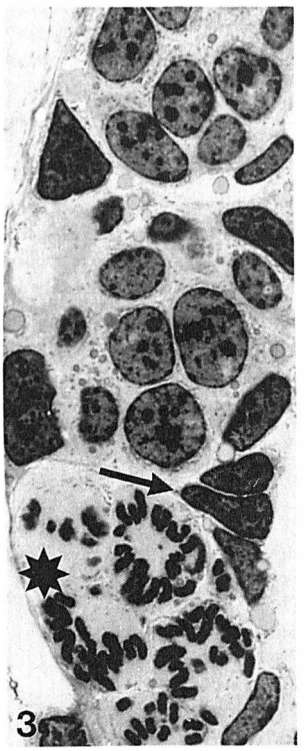

Fig. 3. Zone of secondary spermatogonia in the same testis. The follicular cells (arrow) surrounds groups of secondary spermatogonia. Each cell group is originated from mitoses in primary spermatogonia (star). Toluidine blue. x980.

Weight (body) and volume (testis and germ cells)	Initial controls (Dec. 30)	Final controls (March 30)	Exposure to 16L:8D and 4°C (March 30)	Exposure to 16L:8D and 20°C (March 30)
Body weight	9362±154^{a}	9404±161^{a}	9262±159^{a}	9311±192^{a}
Left testis	90±11^{a}	94±11^{a}	92±9.2^{a}	158±15^{b}
Primary spermatogonia	3.51±0.6^{a}	4.16±0.6^{b}	4.32±0.4^{ba}	3.31±0.3^{a}
Secondary spermatogonia	10.50±1.2^{a}	22.59±2.2^{b}	19.64±3.2^{c}	16.51±2.3^{d}
Primary spermatocytes	-	-	-	33.20±5.5
Round spermatids	-	-	-	59.12±9.8
Elongated spermatids	-	-	-	5.02±1.0
Spermatozoon bundles	67.64±9.3^{a}	59.92±8.1^{a}	61.24±10^{a}	40.22±6.7^{b}
Glandular tissue	8.35±1.2^{a}	7.33±0.7^{a}	6.80±1.0^{a}	0.62±0.1^{b}

TABLE 1. Body weight (mg), left testis volume (mm^{3}), and volume occupied by each germ cell type and glandular tissue (mm^{3}) in newts exposed to natural or long photoperiods and low or mild temperatures for three months during the period of testicular quiescence. Values are expressed as means ± SD. For each parameter, the values coinciding in one or more superscript letters do not differ significantly; and the values with completely different superscript letters differ significantly (P<0.05). The volume occupied by each germ cell type includes that occupied by their accompanying follicular and interstitial cells. The sample size was eight in each group. ANOVA test was significant for all parameters except for body weigh and primary spermatogonia.

Weight (body) and volume (testis and germ cells)	Initial controls (March 30)	Final controls (June 30)	Exposure to 16L:8D and 4°C (June 30)	Exposure to 16L:8D and 20°C (June 30)
Body weight	9404±161^{a}	9281±161^{a}	9366±186^{a}	9164±170^{a}
Left testis	94±9.3^{a}	139±16^{a}	75±8.2^{c}	216±18^{d}
Primary spermatogonia	4.16±0.6^{a}	4.15±0.6^{a}	3.76±0.7^{a}	3.81±0.6^{a}
Secondary spermatogonia	22.59±2.2^{a}	37.82±3.9^{a}	23.49±3.1^{ac}	25.23±2.9^{c}
Primary spermatocytes	-	58.57±8.3^{a}	-	78.87±9.8^{b}
Round spermatids	-	37.06±4.2^{a}	-	80.11±10^{b}
Elongated spermatids	-	-	-	2.89±0.4
Spermatozoon bundles	59.92±8.1^{a}	-	39.40±6.2^{b}	23.06±3.2^{c}
Glandular tissue	7.33±0.7^{a}	1.40±0.2^{b}	6.80±1.1.2^{a}	2.03±0.5^{c}

TABLE 2. Body weight (mg), left testis volume (mm^{3}), and volume occupied by each germ cell type and glandular tissue (mm^{3}) in newts exposed to natural or long photoperiods and low or mild temperatures for three months during the period of germ cell proliferation and development to round spermatids. Values are expressed as means ± SD. For each parameter, the values coinciding in one or more superscript letters do not differ significantly; and the values with completely different superscript letters differ significantly (P<0.05). The volume occupied by each germ cell type includes that occupied by their accompanying follicular and interstitial cells. The sample size was eight in each group. ANOVA test was significant for all parameters except for body weight and primary spermatogonia.

Weight (body) and volume (testis and germ cells)	Initial controls (March 30)	Final controls (June 30)	Exposure to 16L:8D and 4°C (June 30)	Exposure to 16L:8D and 20°C (June 30)
Body weight	9281±145^{a}	9305±182^{a}	9199±157^{a}	9387±163^{a}
Left testis	139±16^{a}	215±27^{b}	131±16^{a}	210±22^{b}
Primary spermatogonia	4.15±0.6^{a}	3.28±0.7^{b}	3.92±0.4^{a}	5.31±0.8^{c}
Secondary spermatogonia	37.82±3.9^{ab}	43.13±5.1^{c}	38.26±4.3^{bc}	34.41±5.6^{a}
Primary spermatocytes	58.57±8.3^{a}	5.17±0.7^{b}	66.85±7.9^{a}	6.08±0.9^{c}
Round spermatids	37.06±4.2^{a}	8.71±1.1^{b}	21.01±2.9^{c}	9.32±1.3^{b}
Elongated spermatids	-	16.16±2.3^{a}	-	15.71±2.0^{a}
Spermatozoon bundles	-	134.62±18^{a}	-	136.01±18^{a}
Glandular tissue	1.40±0.2^{a}	3.93±0.5^{b}	0.96±0.4^{c}	3.16±0.5^{d}

TABLE 3. Body weight (mg), left testis volume (mm^{3}), and volume occupied by each germ cell type and glandular tissue (mm^{3}) in newts exposed to natural or long photoperiods and low or mild temperatures for three months during the period of spermiogenesis. Values are expressed as means ± SD. For each parameter, the values coinciding in one or more superscript letters do not differ significantly; and the values with completely different superscript letters differ significantly (P<0.05). The volume occupied by each germ cell type includes that occupied by their accompanying follicular and interstitial cells. The sample size was eight in each group. ANOVA test was significant for all parameters except for body weight and secondary spermatogonia.

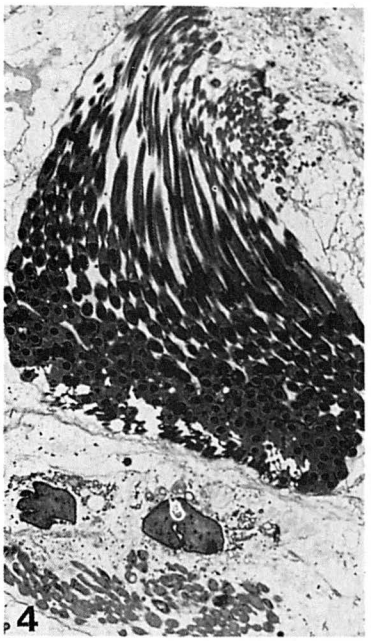

Fig. 4. Spermatozoon bundle in the same testis. Toluidine blue. x580.

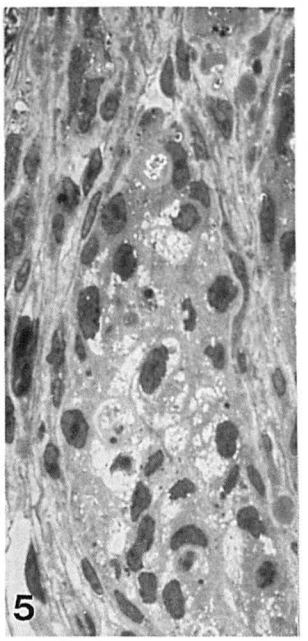

Fig. 5 Glandular tissue in the same testis. Toluidine blue. x365.

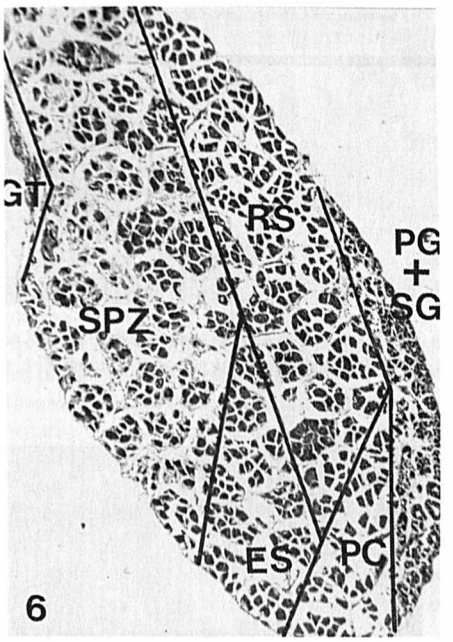

Fig. 6. Testicular lobe from a marbled newt exposed to 20°C and 16L:8D for three months during the quiescent period. The distribution of germ cell zones is indicated by black lines. PC: primary spermatocytes; RS: round spermatids; ES: elongated spermatids; for the other letters see fig. 1. Haematoxylin and eosin. x22.

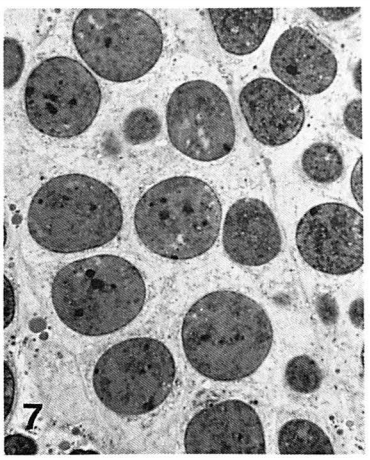

Fig. 7. Zone of primary spermatocytes in the contralateral testis from the same newt. Toluidine blue. x700.

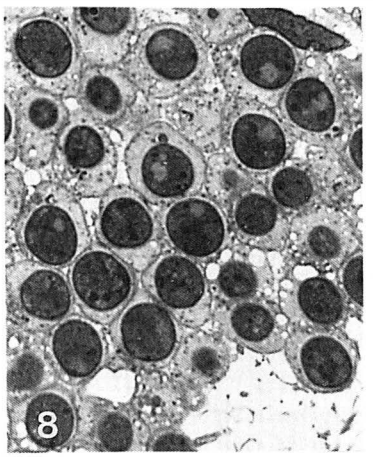

Fig. 8. Zone of round spermatids in the same testis. Toluidine blue. x790.

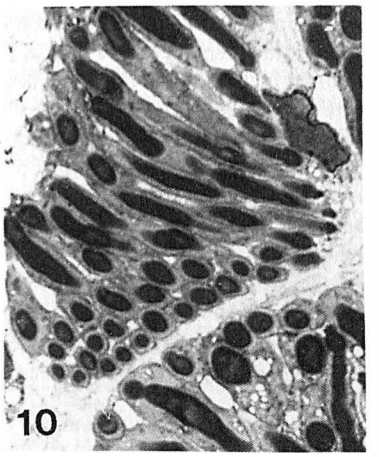

Fig. 10. Elongated spermatids in the same testis. Toluidine blue. x770.

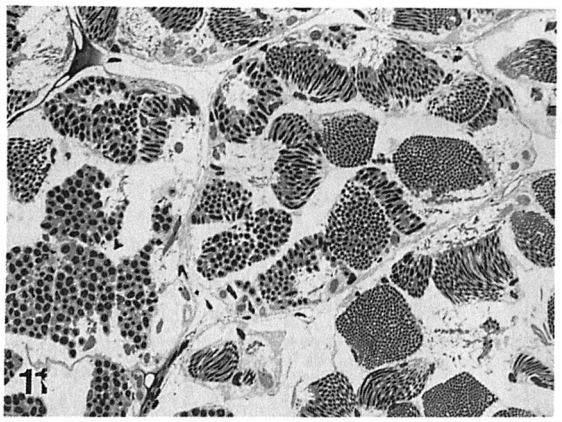

Fig. 11. Spermiogenesis in the testis of a final control newt sacrificed at the end of the spermiogenesis period. Toluidine blue. x115.

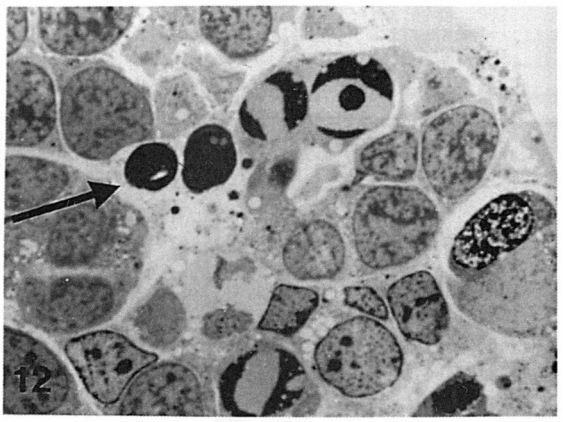

Fig. 12. Degenerating (arrow) spermatocytes in the testis of a marbled newt maintained at 4°C and 16L:8D for three months during the period of spermiogenesis. Toluidine blue. x630.

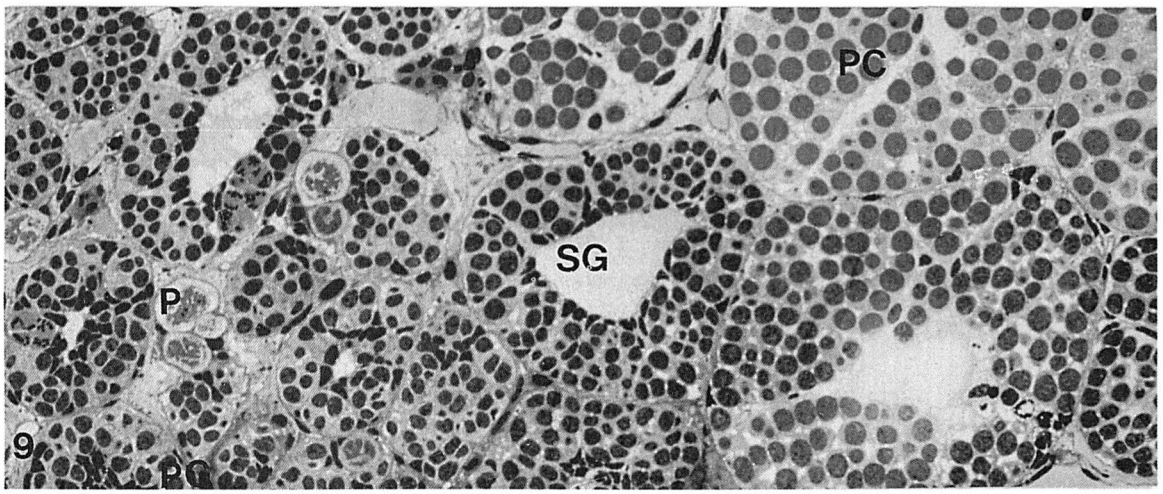

Fig. 9. Part of a testicular lobe from a final control newt sacrificed at the end of the germ cell proliferation period showing primordial germ cells (P), primary (PG) and secondary (SG) spermatogonia, and spermatocytes (PC). Toluidine blue. x200.

RESULTS

Period of Testicular Quiescence (January-March)

No significant differences between the initial controls, final controls, and newts maintained at 4°C were found for any of the parameters measured (Table 1). No spermatogenesis occurred in these newts (Fig. 1). The germ cell types present were primordial germ cells, primary (Fig. 2) and secondary spermatogonia (Fig. 3) and spermatozoon bundles (Fig. 4), these and the glandular tissue (Fig. 5) were probably formed in the preceding cycle (Sáez et al., 1990). The newts maintained at 20°C showed larger testes and developed spermatogenesis up to the level of round spermatids and a few elongated spermatids (Figs. 6-8). The volume occupied by the glandular tissue and that occupied by spermatozoon bundles were lower than in the other groups, suggesting a certain level of spermatozoon release (Table 1).

Period of Germ Cell Proliferation up to Round Spermatids (April-June)

The last controls presented larger testes than the first ones, germ cell development up to round spermatids level, and scanty glandular tissue (Fig. 9). Spermatogenic development was more marked in the newts exposed to 20°C (Table 2). These animals had elongated spermatids (Fig. 10) and their spermatozoon bundles were probably formed during the experiment. The testes of the newts exposed to 4°C were similar to those of the first controls although the former testes were smaller in size and contained less spermatozoon bundles (Table 2).

Period of Spermiogenesis (July-September)

The final control as well as the newts exposed to 20°C achieved spermatogenesis (Fig. 11) and their glandular tissue was increased. The newts exposed to 4°C did not develop spermiogenesis and were similar to the initial controls except for a decrease in the volume occupied by the round spermatids (Table 3). Vacuolated cells with pyknotic nuclei corresponding to degenerating primary spermatocytes or round spermatids could be observed in the newts kept at 4°C (Fig. 12).

DISCUSSION

The results of this study indicate that, during the phase of testicular quiescence, long photoperiod failed to induce spermatocyte formation at cold temperatures (4°C). Since spermatocytes develop in marbled newts (Fraile *et al.*, 1988)

and several other urodele species when they are exposed to short photoperiods and mild temperatures (20°C) during this phase of the cycle (Werner, 1969; Steinborn, 1984), it would seem that temperature, and not photoperiod, induces spermatocyte formation in these urodeles. The observation of spermatocytes in several urodele species maintained at 10-11°C and short photoperiods during the quiescent phase (Werner, 1969; Steinborn, 1984) can be explained on the basis that these temperatures (but not 4°C) are permissive to spermatocyte formation.

Present results show that a temperature of 4°C did not hinder proliferation of secondary spermatogonia during testicular quiescence. In the anuran *Rana esculenta* (Rastogi *et al.*, 1976) and in other anuran species (Lofts, 1974) the environmental winter temperature favours the development of primary spermatogonia while secondary spermatogonia only develop with the mild temperatures in spring.

The effect of temperature on spermatogenic development in amphibians seems to be mediated by the hypothalamic-hypophyseal system (Mazzi, 1970).In the anuran *R. esculenta*, the absence of spermatocyte formation at low temperatures seems to be caused by the suppression of gonadotropin secretion by the hypophysis and not by decreased activity of gonadotropin receptors in the germinal epithelium. In this species the germinal epithelium is sensitive to gonadotropin stimulation at wide ranges of temperature since gonadotropin administration induces spermatocyte formation at either low or high temperatures (Rastogi *et al.*, 1976). In another anuran species, *Rana temporaria*, low temperatures only insensitize the germinal epithelium to gonadotropins during the first part of the quiescent period (van Oordt, 1956; van Oordt & Lofts, 1963). In other anurans (Lofts, 1974) and in the urodele *T. cristatus* (Galgano & Flachetti, 1940) low temperatures inhibit the sensitivity of the germinal epithelium at any time of the year. This latter probably occurs also in the marbled newt and other urodele species; in the red-bellied newt, gonadotropin levels are as high or even higher in the period of quiescence (with low environmental temperatures) as in the period of germ cell proliferation (with mild temperatures) (Tanaka, Hanaoka & Takikawa, 1980; Tanaka, Takikawa & Wakabayashi, 1981). Studies on follicle-stimulating hormone (FSH) receptors in the urodeles *Cynops pyrrhogaster* (Kubokawa & Ishii, 1980) and *Hynobius retardatus* (Kubokawa, Moriya & Ishii, 1985) indicated that the gonadotropin affinity of FSH receptors in these urodeles decreases with low temperature (0°C).

In the annual cycle of the marbled newt, spermatozoon bundles are released from the testis at the beginning of the period of germ cell proliferation (Sáez *et al.*, 1990). The testes of newts exposed to 4°C during the period of germ cell proliferation show the same absence of germ cell development as the initial controls although a certain degree of spermatozoon release occurred. This suggest that spermatozoon release is not secondary to spermatocyte formation. Both temperature and photoperiod might be important factors controlling this process. Ifft (1942) suggested that temperature and not photoperiod controls this process, as spermatozoon release was not observed in the urodele *Notophthalmus viridescens* maintained at 8°C and either total darkness or constant light during the period of cell proliferation. This finding agrees with our previous observation of spermatozoon release in marbled newts exposed to mild temperature (20°C) and short photoperiods during this phase of the cycle (Fraile *et al.*, 1989a). However, in marbled newts maintained at 20°C during the quiescent period, spermatozoon release only occurred in the animals exposed to long photoperiods and only to a certain degree (Fraile *et al.*, 1988). All these findings lead us to suggest that, in addition to temperature, other factors such as photoperiod, a certain degree of spermatogonial proliferation, and the persistence of the glandular tissue are probably involved.

In the testicular cycle of the marbled newt, a pronounced reduction in glandular tissue associated with spermatozoon release is observed in the period of germ cell proliferation. Spermatozoon release seems to be regulated by a decrease in androgen production and an increase in gonadotropin secretion (Lofts, 1974). Hormone studies on the annual testicular cycle of the newt *Cynops pyrrhogaster* (Tanaka & Takikawa, 1983) indicated that plasma testosterone levels decrease abruptly in spring when spermatocyte proliferation begins. In our study, glandular tissue persisted in the newts exposed to 4°C during this period. However, this does not mean that androgen levels remain high because the abundant glandular tissue present in winter in the natural annual cycle is inactivated by the low temperatures (Tanaka & Takikawa, 1983; Fraile, Paniagua, Sáez & Rodríguez, 1989c; Fraile, Paniagua, Rodríguez, Sáez & Jiménez, 1989d). Therefore, present results suggest that cold temperatures hinder the disappearance of the glandular tissue and inactivate androgen synthesis by this tissue that would otherwise lead to spermatozoon release.

In the marbled newt, exposure to 4°C during the spermiogenesis period impedes spermiogenesis and causes degeneration of spermatids and spermatocytes. This effect seems to be related with temperature and not with photoperiod since spermiogenesis occurs in newts exposed to mild temperatures and either short or long photoperiods (Fraile *et al.*, 1989b). Therefore, temperature seems to regulate not only spermatocyte formation and meiosis but also spermiogenesis. In previous experiments we found that high temperatures (30°C) do not affect spermatocyte formation and meiosis although they hinder spermiogenesis (Fraile *et al.*, 1989). The negative effects of cold temperatures on spermiogenesis might be mediated by androgen secretion. In mammals (Ritzén, Biotani, Parvinen, French & Feldman, 1982; Parvinen & Ruokonen, 1982; Paniagua, Rodríguez, Nistal, Fraile, Amat & Regadera, 1986) and in some anuran species (Rastogi, Tammaro, di Meglio, Iela, di Matteo & Chieffi, 1981) spermatid differentiation is a directly androgen-dependent process. In many urodeles species (Ucci, 1982; Pudney & Callard, 1984; Lecoteaux, Garnier, Bassez, Joly, 1985) including the marbled newt (Fraile et al., 1990), spermatozoon formation precedes glandular tissue formation and the subsequent increase in androgen synthesis. Therefore, spermiogenesis in urodeles does not seem to be androgen-dependent although it might be mediated by the increase in the gonadotropin levels observed at the beginning of the spermatogenesis period (Tanaka *et al.*, 1980). As has been said for spermatocyte formation, the negative effects of low temperatures on spermiogenesis in the marbled newt might be related with spermatid insensitivity to gonadotropins.

At the end of the spermiogenesis period the glandular tissue increases again in the marbled newts exposed to mild temperatures but not in the animals maintained at 4°C. In previous experiments we have shown that photoperiod does not influence glandular tissue development in the marbled newt (Fraile *et al.*, 1989b) while high temperatures hinder glandular tissue formation (Fraile *et al.*, 1989c). In many urodele species (Specker &

Moore, 1980; Tanaka & Takikawa, 1983; Imai, Tanaka & Takikawa, 1985; Fraile *et al.*, 1989d) the favourable temperatures for testosterone synthesis are comprised within a narrow range (the mild temperatures of early spring and late autumn). However, the role of cold temperatures in the lack of glandular tissue development in the spermiogenesis period might be secondary to the lack of spermiogenesis since transformation of interstitial peritubular cells into Leydig cells only occurs in the cysts in which spermiogenesis is completed (Fraile *et al.*, 1990).

The results of the present and previous studies on external factors controlling testicular function in newts suggest: (1) neither temperature nor photoperiod is necessary for spermatogonial proliferation; (2) mild or high (up to 30°) temperatures are a necessary and sufficient condition for spermatocyte formation; long photoperiods are not necessary; (3) these temperature are a necessary but insufficient condition for meiosis; long photoperiods are also necessary; and development of the glandular tissue; high temperatures hinder these processes while photoperiod has no influence.

ACKNOWLEDGMENTS

This work was partially supported by a grant from the "Dirección General de Investigación Científica y Técnica" (PB89-203). We are indebted to Mr. Antonio Priego for technical assistance. We thank to Mrs. Carrol Warren from the ICE of the University of Alcalá de Henares for linguistic assistance.

REFERENCES

Cei, J. G. M. (1944). Analysis biogeografica e richerche biologiche e sperimentali sul ciclo annuo della rane rosse d'Europa. *Mon. Zool. Ital.* **43**, 156-160.

Fraile, B., Paniagua, R. & Rodríguez, M. C. (1988). Long day photoperiods and temperature of 20°C induces spermatogenesis in blinded and not blinded marbled newts during the period of testicular quiescence. *Biol. Reprod.* **39**, 649-655.

Fraile, B., Paniagua, R., Rodríguez, M. C. & Sáez, F. J. (1989a). Effect of photoperiod on testicular function in blinded and not blinded marbled newts, *Triturus marmoratus* (Caudata, Salamandridae). *J. Morphol.* **200**, 151-162.

Fraile, B., Paniagua, R., Rodríguez, M. C. & Sáez, F. J. (1989b). Effects of photoperiod and temperature on spermiogenesis in marbled newts (*Triturus marmoratus marmoratus*). *Copeia* **1989**, 357-363.

Fraile, B., Paniagua, R., Sáez, F. J. & Rodríguez, M. C. (1989c). Effects of moderately high temperature on the testis of the marbled newt, *Triturus marmoratus*. *Amphibia-Reptilia* **10**, 117-124.

Fraile, B., Paniagua, R., Rodríguez, M. C., Sáez, F. J. & Jiménez, A. (1989d). Annual changes in the number, testosterone content, and ultrastructure of glandular tissue cells of the testis in the marbled newt, *Triturus marmoratus*. *J. Anat.* **167**, 85-94.

Fraile, B., Sáez, F. J. & Paniagua, R. (1990). The cycle of follicular and interstitial cells (Leydig cells) in the testis of the marbled newt, *Triturus marmoratus* (Caudata, Salamandridae). *J. Morphol.* **204**, 89-101.

Galgano, M. & Flachetti, L. (1940). L'influenza della temperatura sulla spermatogenesi e sopra i caratteri sessuabli di *Triton cristatus* Laur. *Mon. Zool. Ital.* **51**, 166-181.

Ifft, J. D. (1942). The effects of environmental factors on the sperm cycle of *Triturus viridiscens*. *Biol. Bull.* **83**, 111-128.

Imai, K., Tanaka, S. & Takikawa, H. (1985). Annual cycle of the gonadotropin and testicular steroid hormones in the Japanese red-bellied newt. In *Current Trends in Comparative Endocrinology*, 247-249. Lofts, B & Holmes, W.N. (Eds). Hong Kong: Hong Kong University Press.

Kubokawa, K. & Ishii, S. (1980). Follicle stimulating hormone (FSH) reception in the testis of the newt, *Cynops pyrrhogaster*, and comparison of temperature dependance of the receptors with those of the other vertebrates. *Gen. Comp. Endocrinol.* **40**, 435-433.

Kubokawa, K., Moriya, T. & Ishii, S. (1980). FSH receptors in the testis of the salamander *Hynobius retardatus*. In *Current Trends in Comparative Endocrinology*, 755-757. Lofts, B. & Holmes, W.N. (Eds). Hong Kong: Hong Kong University Press.

Lecoteaux, A., Garnier, D. H, Bassez, T. & Joly, J. (1985). Seasonal variations of androgens, estrogens, and progesterone in the different lobules of the testis and in the plasma of *Salamandra salamandra*. *Gen. Comp. Endocrinol.* **58**, 211-221.

Lofts, B. (1974). Reproduction. In *Physiology of the Amphibia*. Vol. 1, 197-218. Lofts, B. (Ed). New York: Academic Press.

Mazzi, V. (1970). The hypothalamus as a thermodependent neuroendocrine center in urodeles. In *The Hypothalamus*, 663-676. Martini, L., Motta, M. & Fraschini, F. (Eds). New York: Academic Press.

Paniagua, R., Rodríguez, M. C., Nistal, M., Fraile, B., Amat, P. & Regadera, J. (1988). Changes in surface area and number of Leydig cells in relation to the 6 stages of the cycle of the human seminiferous epithelium. *Anat. Embryol.* **178**, 423-427.

Parvinen, M. & Ruokonen, A. (1982). Endogenous steroids in the rat seminiferous tubules. Comparison of the stages of the epithelial cycle isolated by transillumination-assisted microdissection. *J. Androl.* **3**, 211-220.

Pudney, J. & Callard, G. V. (1984). Organization of interstitial tissue of the salamander *Necturus maculosus* (Caudata: Proteidae). *J. Morphol.* **181**, 87-95.

Rastogi, R. K., Iela, L., Sasena, P. K. & Chieffi, G. (1976). The control of spermatogenesis in the green frog, *Rana esculenta*. *J. Exp. Zool.* **196**, 151-166.

Rastogi, R. K., Tammaro, L., di Meglio, M., Iela, L., di Matteo, L. & Chieffi, G. (1981). Circannual testicular rhythm in the green frog *Rana esculenta*. *Boll. Zool.* **48**, 97-105.

Ritzén, E. M., Biotani, C., Parvinen, M., French, F. S. & Feldman, M. (1982). Stage dependent secretion of ABP by rat seminiferous tubules. *Mol. Cell. Endocrinol.* **22**, 25-33.

Rouy, S. (1972). Observations sur la structure du testicle adulte chez l'urodele pyrénéen *Euproctus asper* Dúgesetses, variations au course du cycle sexuel chez une population de basse altitude. *Bull. Soc. Zool. Fr.* **97**, 653-674.

Sáez, F. J., Fraile, B. & Paniagua, R. (1990). Histological and quantitative changes on the annual testicular cycle of *Triturus marmoratus marmoratus*. *Can. J. Zool.* **68**, 63-72.

Specker, J. L. & Moore F. L. (1980). Annual cycle of plasma androgens and testicular composition in the rough-skinned newt, *Taricha granulosa*. *Gen. Comp. Endocrinol.* **42**, 297-303.

Steinborn, J. K. (1984). Untersuchungen zur Jahresperiodik der Fortpflanzung beim mÑnnchlichem Kammolch, *Triturus cristatus* Laurenti, in Abhangigkeit von Photoperiode und Wassentemperatur. *Amphibia-Reptilia* **5**, 231-243.

Tanaka, S. & Takikawa, H. (1983). Seasonal changes in plasma testosterone and 5α-dihydrotestosterone levels in the adult male newt, *Cynops pyrrhogaster pyrrhogaster*. *Endocrinol. Jpn.* **30**, 1-6.

Tanaka, S., Hanaoka, Y. & Takikawa, H. (1980). Seasonal changes in gonadotropin activity of the pituitary gland in the adult male Japanese red-bellied newt, *Cynops pyrrhogaster pyrrhogaster*. *Zool. Magazine* **89**, 187-191.

Tanaka, S., Takikawa, H. & Wakabayashi K. (1981). Seasonal variations in pituitary gonadotropins in the adult male newt, *Cynops pyrrhogaster pyrrhogaster*, revealed by isoelectric focusing technique and radioreceptor assay. *Endocrinol. Jpn.* **28**, 335-345.

Ueci, A. A. (1982). A fine-structural study of interstitial cell changes in the testes of *Necturus maculosus* during a portion of the annual cycle, and possible evidence for local feed back control by seminiferous epithelium. *Am. J. Anat.* **165**, 27-38.

van Oordt, P. G. W. (1956). The role of temperature in regulating the spermatogenetic cycle in the common frog, *Rana temporaria*. *Acta Endocrinol.* **23**, 251-264.

van Oordt, P. G. W. & Lofts, B. (1963). The effect of high temperature on gonadotropin secretion in the male common frog (*Rana temporaria*) during autumn. *J. Endocrinol.* **27**, 137-146.

Werner, J. K. (1969). Temperature-photoperiod effects on spermatogenesis in the salamander *Plethodon cinereus*. *Copeia* **1969**, 592-602.