mean fiber diameter, the muscle fibers were in the 30–45 µm range (see fig. 1B).

Figure 2 is a composite of examples of electrophysiologic recordings made from the sternothyroid muscle. Under the conditions described in the methods section, resting potentials between −65 and −78 mV were readily obtained. Figure 2A and B are examples of miniature end-plate potentials (MEPPs) recorded intracellularly and extracellularly, respectively. The mean MEPP amplitude (corrected to a resting potential of −90 mV) recorded intracellularly was 0.71 mV. Figure 2C shows an intracellularly recorded action potential resulting from indirect stimulation via the ansa hypoglossus nerve.

Discussion. Because the rat sternothyroid muscle is considerably thicker than the mouse omohyoid muscle, it is probably unsuitable for use with Normarski optics, except at the lateral edges where it is only a few fibers thick. Nevertheless, the ease of localizing end-plates within the rat sternothyroid muscle, as described, is a great advantage. Localization of end-plate regions for electronmicroscopic evaluation has always presented a problem. Engel's method[8] for locating end-plates consists of cutting fixed muscle into 2 strips; reacting one strip with acetylcholinesterase stain; embedding the reacted and unreacted strips parallel to each other; viewing both strips in the dissection microscope; and removing and reembedding the region in the unreacted muscle strip which corresponds to the stained end-plate region in the reacted strip. Since the 'motor point' (zone where end-plates are concentrated) in the sternothyroid muscle is located adjacent to the readily visualized nerve which innervates it, the sternothyroid 'motor point' is readily excised by cutting along each side of the nerve. This technique has provided us with ample end-plates for electronmicroscopic evaluation[9] without having to employ the more cumbersome and time-consuming method described by Engel[8]. With practice, the dissection can be accomplished in 5–10 min. This coupled with the ability to visualize the end-plate region and visually control impalements also makes the preparation very suitable for electrophysiological studies.

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Leydig cell and Δ^5-3β-hydroxysteroid dehydrogenase activity in the testis of toad *(Bufo melanostictus)* following cold exposure

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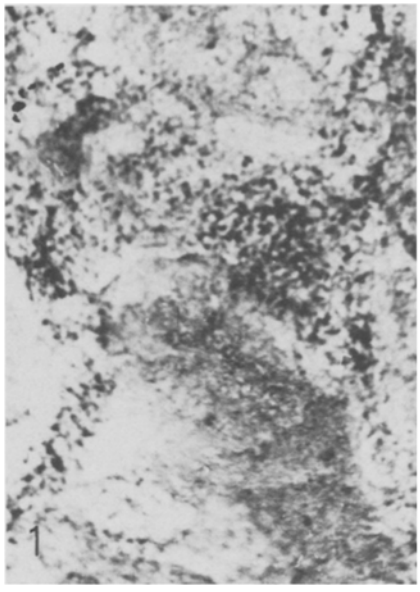
Summary. Leydig cell nuclear area and Δ^5-3β-hydroxysteroid dehydrogenase activity were increased in the testis of *Bufo melanostictus* 2 days after a single short-term cold exposure. Both parameters returned to normal values 4 days later.

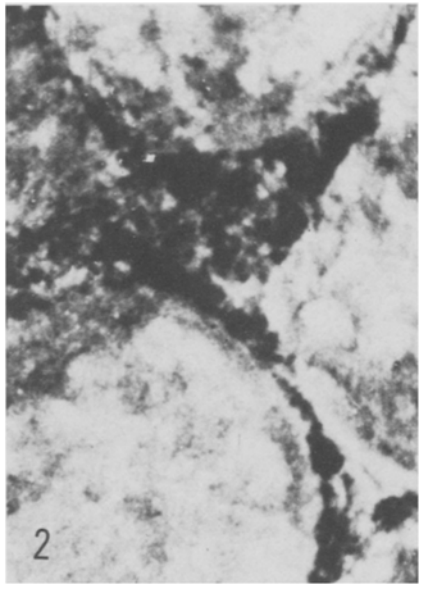
The influence of ambient temperature on testicular activity in different classes of vertebrates is well established[1-4]. Compared with heat, cold is much less severe in its effect on testicular function of mammals and much greater extremes are required to produce an effect in higher vertebrates[5,6]. However, little is known about the influence of severe hypothermia on the amphibian testis. In view of the above findings the present study was undertaken to elucidate whether severe cold exposure can alter the testicular endocrine activity in amphibia. Testicular steroidogenic activity in *Bufo melanostictus* was evaluated mainly by demonstrating histochemically Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSD), a key enzyme of steroid hormone production in both mammalian and non-mammalian vertebrates[7-8].

Materials and methods. 70 male toads of average body weight 56 g were used in the present experiment. All the animals were collected from their natural habitat during the breeding season (June/July). They were kept in the laboratory for a few days prior to experimentation in the presence of water and normal periods of light and darkness. Ant eggs were supplied as food on alternate days. 40 toads were exposed to cold in a cold chamber at −2 °C for 3 h. At the end of the exposure they were taken out of the chamber for revival. 75% of the exposed toads revived. The animals were divided equally into 3 groups. Group A: unexposed control animals; group B: exposed toads sacrificed 2 days after cold exposure; group C: exposed toads sacrificed 6 days after cold exposure; group D: exposed toads sacrificed 10 days after cold exposure.

The animals were sacrificed according to their scheduled date of sacrifice along with controls. Fresh frozen sections from 1 testis were cut at 20 µm on a cryostat at −20 °C for histochemical demonstration of Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSD) using dehydroepiandrosterone as substrate[9]. Control sections were incubated simultaneously for the same time period in a medium containing no substrate. After incubation, sections were fixed in 10% formol, washed in distilled water, dried and mounted in glycerine jelly. Another testis was fixed in Bouin's fluid for histometric studies of Leydig cell nuclear area (LCNA) according to Deb at al.[10]. For histology, 5-µm sections were prepared and stained with hematoxylin-eosin.

Results and discussion. Δ^5-3β-HSD activity was localized





Leydig cells stained for Δ^5-3β-HSD activity. Figure 1. Control. Figure 2. 2 days after short term exposure at −2 °C. ×250.

in the Leydig cells of the control testis (fig. 1). Enzyme activity appeared to be markedly elevated in testicular slices from group B animals (fig. 2) in comparison with the control testis. Δ^5-3β-HSD activity decreased in group C and D animals in comparison with group B and the activity of the enzyme returned to control level.

Histometric studies of Leydig cell nuclear area (LCNA) showed that LCNA increased significantly 2 days after exposure. LCNA was decreased in group C as compared to group B. In group D, LCNA returned more or less to the control value (table).

The present study demonstrates that on the 3rd day after cold exposure Δ^5-3β-HSD and Leydig cell nuclear area (LCNA) increased significantly. Since this enzyme is concerned with steroid hormone synthesis[8–10], and Leydig cell nuclear area (LCNA) reflects the overall androgen biosynthesis status of the testis[11], increases in Δ^5-3β-HSD activity and of LCNA strongly suggest an augmentation of hormone production after cold exposure. After 6 days of cold exposure Δ^5-3β-HSD activity and LCNA appear to return to normal. The probable mechanism of this cold-induced alteration of hormone production in amphibian testis is not yet clear.

Van Oordt and de Kort[12,13] showed that the cooler months of the year favor an interstitial cell response to circulating gonadotropins and in this connection it has been reported earlier that in *Rana temporaria* and *R. esculenta*[14,15], Leydig cell activity is high during the autumn. De Kort[13] observed a greater sensitivity and stimulation of the interstitial tissue to LH treatment in *Rana esculenta* at the lower temperature.

A significant rise of the serum LH level has been reported under conditions of stress induced by cold[16]. Since LH controls the nuclear area of the Leydig cells and Δ^5-3β-HSD activity[17,18], the present experimental results indicate that the enhancement of LCNA and Δ^5-3β-HSD activity is possibly due to a rise of pituitary LH secretion as well as an increased sensitivity of Leydig cells to LH in toads exposed to cold stress.

Leydig cell nuclear area (mm², camera lucida × 800) of toads testis following cold exposure

Control (group A) (6)	2 days after cold exposure (group B) (6)	6 days after cold exposure (group C) (6)	10 days after cold exposure (group D) (6)
21.32 ± 0.47	34.51 ± 3.61*	20.28 ± 0.80	21.74 ± 0.82

Level of significance (Calculated on the basis of Student's t-test)

A vs B $p < 0.01$
B vs C $p < 0.01$
B vs D $p < 0.01$

Values are means ± SEM. Figure in parenthesis indicates number of animals. *Statistically significant.

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