Failure of the Pineal Removal to Prevent some Cold-Induced Testicular Changes in Golden Hamsters

Morphological and biochemical studies have indicated that the mammalian pineal gland is involved in the regulation of reproductive functions[1-3]. This organ serves as a neurochemical transducer which converts changes in environmental lighting to chemical messages and thus is able to regulate the reproductive process[4]. Study of the role of the pineal gland in environmental influences on reproduction has been approached mainly from the standpoint of lighting. Little is known about the gland in relation to low temperatures. Hibernators such as hamsters are of particular interest. The reproductive organs of these animals, in contrast to those of non-hibernators such as rat[5,6], are sensitive not only to environmental lighting but also to cold. Exposure of male hamsters to cold, 5 or 6°C, resulted in regression of testes within 8 weeks even though a long photoperiod was provided[7,8]. However, it is not known how the effect of cold is mediated. The purpose of this study was to determine whether the pineal is involved.

Materials and methods. 102 adult male golden hamsters weighing 80–90 g were purchased from the Con Olson Co., Madison, Wisconsin. These animals were caged individually with a minimum amount of shredded paper to avoid excessive nest building, and were given Purina Laboratory Chow and water ad libitum. The animals were divided into 6 groups: 1. room temperature (25 °C), non-operated, 2. room temperature, sham-operated, 3. room temperature, pinealectomized, 4. cold-exposed (5 °C), non-operated, 5. cold-exposed, sham-operated, and 6. cold-exposed, pinealectomized. Prior to experiments, all animals were kept in 14 h light: 10 h darkness cycles at room temperature for 2 weeks. Pinealectomies were performed under nembutal anesthesia by the method of HOFFMAN and REITER[9]. After the operation, the animals were given oxytetracycline hydrochloride in drinking water and allowed to recover for 1 week. Six or 5 animals from each group were killed at 4, 8 and 12 weeks and the skull was opened to verify that pinealectomy was complete. The testes were removed from each animal and weighed and one organ was fixed in 10% neutral formalin, embedded in paraffin, sectioned at 4 μm, and stained by the PAS-hematoxylin technique. A portion of the remaining testis was weighed using a Mettler Analytical Balance and dried for 14–16 days to a constant weight ($\pm$ 2 mg) in an electric oven at 60 $\pm$ 1 °C for determination of water content and dry weight[10]. A second portion of the testis was analyzed for nitrogen content using the micro-Kjeldahl method[11].

Result and discussion. In order to avoid any seasonal effect on reproductive functions, the study was conducted between early May and early August, a time when reproductive activity of hamsters is maximal in the field[12]. Except in pinealectomized animals maintained at room temperature for 12 weeks where testes weights were decreased as compared with those of the 2 non-pinealectomized groups, no difference in testes weight was found between different groups of animals kept at room temperature (Table). As in a previous study[8], testes weight was decreased in cold-exposed animals as compared with that of the room temperature animals. No difference in testes weight, expressed either as mg or mg/100 g body weight, was observed in pinealectomized and non-pinealectomized cold-exposed animals at the 3 intervals studied. Histological observation revealed that spermatogenesis was normal in all animals maintained at room temperature (Figure 1). In contrast, sper-

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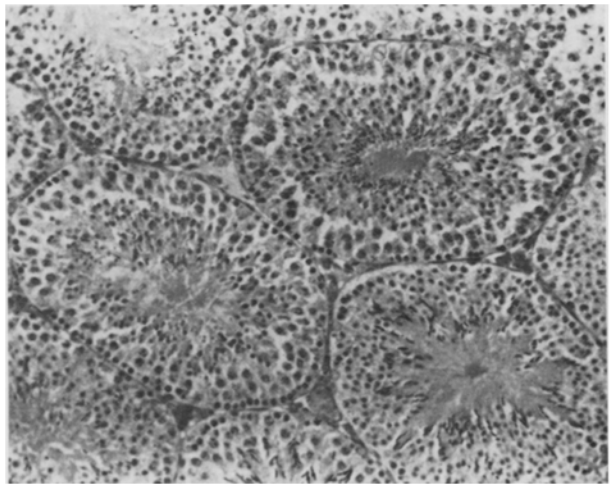

Fig. 1. Cross section of testis from a 4-week non-operated room control hamster showing a normal spermatogenesis. × 150.

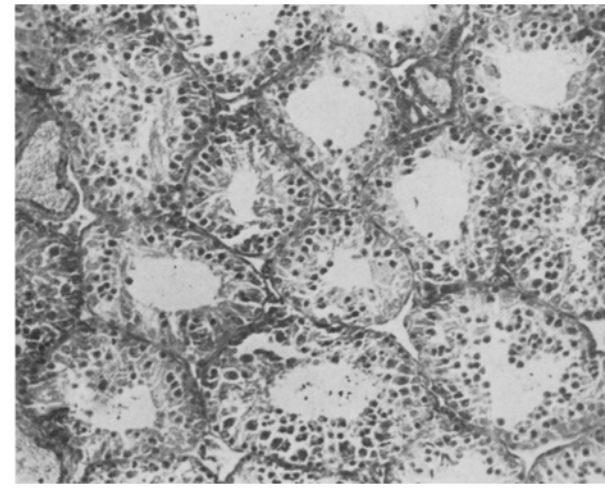

Fig. 2. Cross section of testis from a 4-week non-operated cold-exposed hamster. Note the absence of mature spermatids or spermatozoa in most of the seminiferous tubules. × 150.

Effect of pinealectomy on testes of cold-exposed and control hamsters (M ± S.E.) on a 14-hour photoperiod

Weeks		Treatment	No. of animals	Testes weight mg	Testes weight mg/100 g	dry weight %	N %
4 Cold	Room	Control	6	[a]3109 ± 173	3184 ± 116	15.2 ± 1.4	1.50 ± 0.01
		Sham	6	3242 ± 70	3190 ± 73	13.9 ± 0.1	1.52 ± 0.01
		Pnx[b]	6	3116 ± 61	3174 ± 84	14.0 ± 0.1	1.54 ± 0.02
		Control	6	2252 ± 265	2333 ± 299	14.0 ± 0.1	1.56 ± 0.01
		Sham	6	2245 ± 427	2318 ± 414	14.4 ± 0.2	1.52 ± 0.02
		Pnx	6	1944 ± 575	2006 ± 554	14.7 ± 0.5	1.47 ± 0.07
8 Cold	Room	Control	6	3166 ± 126	3779 ± 241	13.8 ± 0.1	1.53 ± 0.01
		Sham	6	3108 ± 135	3625 ± 183	13.9 ± 0.1	1.53 ± 0.01
		Pnx	6	3268 ± 201	3885 ± 228	14.0 ± 0.1	1,55 ± 0.01
		Control	6	1641 ± 373	1875 ± 375	14.0 ± 0.1	1.60 ± 0.03
		Sham	6	1794 ± 440	2104 ± 480	14.0 ± 0.4	1.60 ± 0.03
		Pnx	6	2098 ± 424	2486 ± 465	14.2 ± 0.3	1.61 ± 0.04
12 Cold	Room	Control	5	3042 ± 163	3101 ± 72	13.9 ± 0.1	1.53 ± 0.01
		Sham	5	3290 ± 173	2904 ± 170	14.0 ± 0.1	1.54 ± 0.01
		Pnx	5	2717 ± 88	2657 ± 57	13.9 ± 0.1	1.52 ± 0.02
		Control	5	2257 ± 455	2330 ± 435	14.4 ± 0.1	1.60 ± 0.03
		Sham	5	2483 ± 255	2546 ± 209	14.3 ± 0.1	1.63 ± 0.01
		Pnx	5	1968 ± 293	2227 ± 376	14.5 ± 0.1	1.66 ± 0.01

[a] Arrows indicate significant difference between groups ($P < 0.05$). [b] Pnx, pinealectomized.

matogenesis was affected in all 3 groups of cold-exposed animals. The number of testes affected to the total number of testes observed for control, sham-operated and pinealectomized hamsters was $^1/_6$, $^3/_6$ and $^3/_6$ respectively at 4 weeks, $^3/_6$, $^2/_6$ and $^2/_6$ respectively at 8 weeks, and $^1/_5$, $^0/_5$ and $^1/_5$ respectively at 12 weeks. The figures reveal that at 4 weeks, 33% of the non-pinealectomized animals and 50% of the pinealectomized animals showed histological changes. At 8 weeks, 42% of the non-pinealectomized and 33% of the pinealectomized showed similar changes. At 12 weeks, the percentages were 10 and 20, respectively. It is clear that pinealectomy did not prevent inhibition of spermatogenesis by cold since as many pinealectomized animals were affected as non-pinealectomized animals at 4,8 and 12 weeks. In the affected testes, spermatogenesis in most cases proceeded up to young spermatid stage or pachytene spermatocyte stage (Figures 2 and 3), although in some seminiferous tubules only Sertoli cells and spermatogonia were present. Only minor changes in per cent dry weight, percent water and percent nitrogen content of the testes had occurred. Dry weight and nitrogen content of the testis were slightly greater, and consequently water content correspondingly lower, in the 4 and 12 week cold-exposed hamsters. There were no differences in these values between pinealectomized and nonpinealectomized animals.

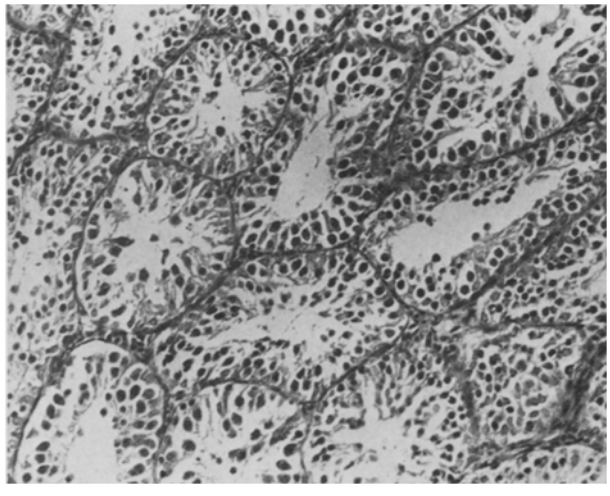

Fig. 3. Cross section of testis from a 4-week pinealectomized cold-exposed hamster. Note the similar appearance of seminiferous tubules as compared with Figure 2. ×150.

As stated earlier, a decrease in weight of the testes and arrested spermatogenesis was observed in pinealectomized and non-pinealectomized cold-exposed hamsters. On the basis of weight changes and histological observations, as well as water and nitrogen content of testes, it would seem that pinealectomy does not prevent cold-induced testicular changes or atrophy. These results further support the findings of REITER[13] who noted that removal of the pineal did not prevent uterine regression in cold-exposed hamsters which were subjected to 16 h of light per day. It is of interest to note that in another study[14], lamella structures in the pinealocytes appeared prior to gonadal atrophy, and were found only in cold-exposed hamsters with regressed testes. These results suggested that gonadal atrophy due to cold exposure was mediated by the pineal. Our results do not support this suggestion. The effect of cold on pineal function has also been studied in the rat by MILINE et al.[15–18]. They report an

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increase in the activities of some pineal enzymes (e.g., succinate dehydrogenase and monoamine oxidase) and also other pineal ultrastructural changes (e.g. hypertrophy and hyperplasia of pineocytes) in response to cold exposure. However, no attempt was made in these studies to correlate the above changes with possible alteration in reproductive functions. The activity of monoamine oxidase (MAO) is of interest, since it is involved in the metabolism of serotonin, a pineal indole which is strongly antigonadotropic when administered to the male rat[19-21]. It has been reported that most of the serotonin produced in the pineal is metabolized by MAO[22]. It is possible, therefore, that an increase in metabolism of serotonin by virtue of increased activity of MAO in the cold-exposed rats might account, at least in part, for the failure of cold temperature to affect the testes of these animals[5,6] as reported previously[23].

Résumé. La diminution du poids des testicules et l'arrêt de la spermatogenèse furent observés chez des hamsters exposés au froid et maintenus dans un cycle alternant de 14 h de lumière et 10 h d'obscurité. En enlevant la glande pinéale, ces changements organiques ne se sont pas produits.

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Sherrington was Right About the Trapezius Muscle Innervation

In 1898 SHERRINGTON[1] reported that the Trapezius muscle of the monkey received motor innervation from the upper cervical spinal nerves in addition to motor innervation from the XI cranial nerve. This cervical spinal innervation was confirmed by STRAUS and HOWELL[2] through the electrical stimulation of the cut peripheral cervical nerve stumps which produced 'weak, though definite action' in the Trapezius muscle. Then CORBIN and HARRISON[3] reported that stimulation of the peripheral stumps of the upper 5 cervical nerves never produced a 'visible' contraction. They concluded that the entire motor supply was through the XI cranial nerve and the sensory proprioception was by way of the cervical nerves.

None of the above investigators used electromyography, the most sensitive and positive means of detecting the contraction of a muscle. Using as an experimental animal 7 squirrel monkeys, *Saimiri sciureus*, chosen because they are primates, reasonably inexpensive, and relatively easy to handle, an electromyographic investigation was undertaken to clarify the motor innervation to the Trapezius.

Bipolar fine-wire electrodes[4] were inserted into the superior, middle, and inferior positions of the Trapezius muscles 28 to 56 days following a surgical procedure in which the XI cranial nerve was severed proximal to its communication with the second cervival spinal nerve. Electrical stimulation of the cervical spinal nerves 2, 3, and 4 revealed electromyographic evidence of muscle contraction but not a visible gross contraction of the Trapezius muscle. Subsequent histochemical procedures[5] for phosphorylase activity revealed innervated as well as denervated fibres in the 3 portions of the muscle.

Both the electromyographic and histochemical evidence allow the following conclusions to be drawn: 1. The cervical spinal nerves, 2, 3, and 4 do contribute a small number of motor fibres to the Trapezius muscle. 2. While the entire Trapezius receives motor innervation from the cervical spinal nerves 2, 3, and 4, the superior portion receives a greater proportion of this cervical motor innervation.

Once again SHERRINGTON has demonstrated his ability to stand the test of time and modern instrumentation. His conclusions with regard to the Trapezius innervation are sound despite the occasional reports to the contrary.

Zusammenfassung. Beim Eichhörnchen, *Saimiri sciureus* wurde elektromyographisch gezeigt, dass die Nerven in der Region der Nackenwirbelsäule den Trapeziusmuskel mit innervieren. Der obere Anteil dieser über den Trapezius verteilten Bewegungsinnervationen enthält stärkere Beteiligung durch die Nackenbewegungsinnervation.

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