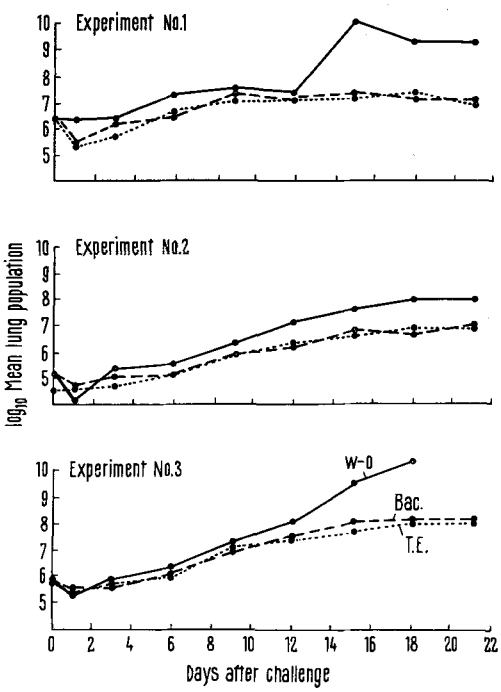

Graphic summaries of increases in the lung populations of challenging tubercle bacilli in nonimmune (w-o), immune (Bac.), and trypsin extract-immunized (T.E.) mice. Data points for experiments 2 and 3 are mean values from triple sets of lungs, but some of those for experiment 1 are for less because contamination interfered with readings on several sample plates.

An added, but related, complication of using minute challenge infections may account for findings[13,16] that mice vaccinated s.c. with BCG appear to have no protection against intrapulmonary multiplication of the challenge bacilli (grown with Tween). We might have seen the same result in our second experiment had we used a somewhat lower challenge infection. Hence, unless tuberculoimmunity is induced by a Tween-associated immunogen applied directly to the lungs, or unless challenge with Tween-grown bacilli suffices to cause progressive bacillary multiplication in unimmunized mice beyond 2 weeks, an acquired immunity actually present may not be detected by Tween-grown challenge bacilli[17].

Zusammenfassung: Extrakte aus Tuberkelbazillen, die mit Trypsin verdaut werden, erzeugen in Mäusen eine Tbc-Immunität, die jener durch BCG entspricht.

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[16] D. W. SMITH, E. WIEGEHAUS, R. NAVALKAR and A. A. GROVER, J. Bact. *91*, 718 (1966).

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The Effect of Pinealectomy and Environmental Lighting on the Gonadal, Thyroid, and Total Body Weight of Female Long-Evans Rats

The mammalian pineal gland has been assigned various possible roles in normal physiology. Dominant among present theories are those which ascribe to this organ a possible hormonal influence upon the gonads[1-6]. The pineal contains large amounts of serotonin and this amine undergoes a circadian rhythm[5,7-10]. Serotonin is converted into melatonin (*N*-acetyl-5-methoxytryptamine) by the enzyme hydroxyindole-*o*-methyl transferase (HIOMT)[2,5,11]. This enzyme is thought to be localized exclusively in the pineal gland in mammals. Activity of this enzyme is thought to be circadian and light influenced, increased in dark and decreased in light. Evidence for this phenomenon has been quite contradictory[2,3,12-14].

Most current theories on pineal control of the gonads have stemmed from the hypothesis that this influence is mediated via pineal melatonin which supposedly acts to inhibit the gonads; melatonin levels are thought to be controlled by environmental lighting through HIOMT activity[2,12,15]. Evidence for this hypothesis has been indirect and is derived from reports that: (1) rats kept in continual light have hypertrophied ovaries while rats kept in total dark have atrophied ovaries[3,4]; (2) melatonin injections cause ovarian atrophy in the rat[16,17] and, (3) ovaries hypertrophy following pinealectomy[1,18].

Because of the pineal-gonadal hypothesis and the various reported interactions between gonads, environmental lighting, and the pineal gland, it was deemed significant to examine gonadal weight in relation to pinealectomy when coupled with variations in environmental lighting conditions. Since it has been reported that pineal melatonin may act to inhibit the secretion of thyroxin[19], the

[1] J. I. KITAY, Endocrinology *54*, 114 (1954).
[2] J. AXELROD, R. J. WURTMAN and S. H. SNYDER, J. biol. Chem. *240*, 949 (1965).
[3] R. J. WURTMAN and J. AXELROD, Scient. Am. *123*, 50 (1965).
[4] R. J. WURTMAN and J. AXELROD, Science *141*, 277 (1963).
[5] W. B. QUAY, Pharmacol. Rev. *17*, 321 (1965).
[6] W. B. QUAY, Progr. Brain Res. *8*, 61 (1964).
[7] W. B. QUAY, Gen. comp. Endocr. *1*, 3 (1963).
[8] W. B. QUAY and A. HALVEY, Physiol. Zool. *35*, 1 (1962).
[9] W. B. QUAY, Z. Zellforsch. *60*, 479 (1963).
[10] W. B. QUAY, Brain Res. *3*, 277 (1967).
[11] S. GERATTINI and L. VALZELLI, *Serotonin* (Elsevier, N.Y. 1965), p. 42.
[12] W. B. QUAY, Physiologist *10*, 286 (1967).
[13] W. B. QUAY, Proc. Soc. expl Biol. Med. *115*, 710 (1964).
[14] W. B. QUAY, Z. Zellforsch. *60*, 479 (1963).
[15] R. COHEN, R. WURTMAN and S. SNYDER, Ann. intern. Med. *61*, 1144 (1964).
[16] W. MCISAACS, R. TABORSKY and G. FARRELL, Science *145*, 63 (1964).
[17] J. IFFT, Endocrinology *71*, 181 (1962).
[18] R. WURTMAN, W. ROTH, M. ALTSCHULE and J. WURTMAN, Acta Endocr. *36*, 617 (1961).
[19] T. ISHIBASHI, D. HAHN, L. SIRVESTAVA, P. KUMARESAN and C. TURNER, Proc. Soc. expl Biol. Med. *122*, 644 (1966).

effect of these parameters upon thyroid weight were also included.

Materials and methods. Adult, female, Long-Evans rats were divided into 3 primary groups. Group I: animals were kept in constant light, group II: animals were kept in total darkness, and group III: animals were kept in diurnal light (12 h light, 12 h dark). Diurnal and continuous light was furnished by 200 watt incandescent bulbs ($= 2.16 \times 10^{-3}$ watts/cm^2). Each primary group was housed in one cage and each group (cage) consisted of pinealectomized animals (7/cage), sham-operated animals (3/cage), and non-operated normal animals (7/cage). Pinealectomy was performed under ether anesthesia. Following surgery environmental lighting was maintained, as described, for 60 days. At the end of this period, animals were sacrificed; total body weight recorded, ovaries, uterus, and thyroid gland were excised, cleaned of connective tissue, and weighed to the nearest milligram with a Torsion balance (model DWL-2). Brains were examined to confirm pinealectomy and to note evidence of brain damage.

Results. No significant difference was noted in thyroid or total body weight among any group of animals, neither when considered as pinealectomized versus normal in any given lighting condition, nor when comparing groups in different lighting conditions (Tables I, II, and III; columns a and d). There was no significant difference between normal and sham-operated animals in any groups; therefore, values from sham-operated animals were incorporated with normal values. In group I animals (continual lighting for 60 days) there was a significant decrease in ovarian weight of pinealectomized animals (Table I, column c). No significant difference was noted in uterine, thyroid, or total body weight between pinealectomized and normal animals (Table I, columns a, b, and d). In group II animals (total darkness for 60 days), there was a significant increase in uterine weight of pinealectomized animals (Table II, column b); however, there was no difference in ovarian, thyroid, or total body weight between pinealectomized and normal animals (Table II, columns a, c, and d). Pinealectomized and normal animals kept in diurnal light for 60 days (group III) showed no difference in any of the measured parameters (Table III, columns a, b, c, and d). However, ovaries of pinealectomized animals kept in normal light were heavier than those of pinealectomized animals kept in continuous light (Tables I and III, column c).

Table I. Continual lighting

	a Total weight	b Uterus weight mg/g	c Ovaries weight µg/g	d Thyroid weight mg/g
Pinealectomy	254.0 g	1.33 ± 0.36	155.0 ± 3.6	1.99 ± 0.12
Normal	257.0 g	1.16 ± 0.38	186.0 ± 4.7	1.74 ± 0.18
Significance (Fischer-*t*-test)		$p > 0.05$	$p < 0.05$	$p > 0.05$

Table II. Continual darkness

	a Total weight	b Uterus weight mg/g	c Ovaries weight µg/g	d Thyroid weight mg/g
Pinealectomy	261.6 g	1.26 ± 0.30	176 ± 2.5	1.72 ± 0.11
Normal	265.2 g	0.97 ± 0.21	187 ± 3.9	1.64 ± 0.18
Significance (Fischer-*t*-test)		$p < 0.05$	$p > 0.05$	$p > 0.05$

Table III. Normal light

	a Total weight	b Uterus weight mg/g	c Ovaries weight µg/g	d Thyroid weight mg/g
Pinealectomy	269.9 g	0.926 ± 0.33	184 ± 3.1	1.68 ± 0.14
Normal	272.0 g	0.99 ± 0.36	190 ± 3.5	1.84 ± 0.26
Significance (Fischer-*t*-test)		$p > 0.05$	$p > 0.05$	$p > 0.05$

Discussion. These results present evidence for a definite pineal gland environmental lighting influence upon gonadal weight. Although one cannot draw absolute correlates between changes in organ weight and functional activity, organ weight changes observed in these experiments do not parallel the functional changes reported elsewhere[3,4,16–18]. In continual light, pinealectomized animals show decreased ovarian weights whereas a functional increase would be expected according to the hypothesized antigonadal effect of pineal melatonin. Normal animals in total darkness show no change in gonadal weight when a functional increase would be expected. Pinealectomized animals in total darkness have an increased uterine weight where one would expect a functional decrease in the ovary.

Conclusions. When pinealectomized and normal animals kept in continual light are compared, a decrease in ovarian weight of the pinealectomized animals is observed. When pinealectomized and normal animals kept in total darkness are compared, an increase in uterine weight of the pinealectomized animals is noted. Pinealectomized and normal animals kept in normal light show no differences in ovarian, uterine, thyroid, or total body weight. However, pinealectomized animals kept in continual light have lighter ovaries than pinealectomized animals kept in normal light.

Weight changes observed in this study do not parallel the functional changes which have been observed in experiments utilizing only pinealectomy or only environmental light as the variable.

Zusammenfassung. Die Ovarien pinealektomierter Tiere weisen bei konstanter starker Beleuchtung ein geringes Gewicht auf, während bei Tieren, die in Dunkelheit gehalten wurden, der Uterus im Vergleich zu Kontrolltieren schwerer ist.

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