Androgenic Activity in the Interscapular Brown Adipose Tissue of the Male Hibernating Bat (*Myotis lucifugus*).* (26182)

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In most mammals the primary and accessory sex gland cycles occur at the same time and the interstitial tissue of the testis is thought to be the source of the androgen promoting this synchronization. In *Corynorhinus* and *Myotis* (see Pearson *et al.*(1) and Miller(2)) there is an apparent dysynchrony between primary and secondary sex glands. In these mammals, spermatogenic activity and interstitial cell and testis size decline rapidly at a time when the accessory glands are undergoing hypertrophy. Accessory glands remain enlarged during the season when testes are shrunken and interstitial cells are small and appear nonsecretory. Pearson *et al.*(1) castrated such animals and found that accessory glands were smaller than noncastrated controls. Nevertheless, histochemical tests to show the presence of androgens and other ketosteroids in the testis of such operated bats were negative. Similar results have been obtained by one of us (P.H.K. unpublished data) in studies on the bat *Myotis lucifugus*. Such information suggests the possibility of the presence of androgen elsewhere in these bats.

Evidence that brown fat of hibernating mammals may contain steroids is offered by Sweet and Hoskins(3) who found, by biological assay, that an alcoholic extract of the "hibernating glands" (interscapular brown fat) of the woodchuck contained the equivalent of 1 International Unit of androgen in 50 g of tissue. These authors suggested that rapid utilization of this tissue following emergence from hibernation, which is coincident with onset of the breeding season, may reflect the part played by the hibernating gland in the sex cycle.

The purpose of our study has been to examine the possibility that there is androgenic activity in the interscapular brown adipose tissue of the male hibernating Vespertilionid bat *Myotis lucifugus*. An effort has been made to determine degree of androgenic activity in this tissue by comparison of seminal vesicle gland growth in weanling rats given various concentrations of testosterone to that of weanling rats receiving a nonsaponifiable fraction of the interscapular brown adipose tissue (NFBF). Results to be reported suggest that interscapular brown adipose tissue provides a notable androgenic stimulus to the target organ (seminal vesicle) in bioassay.

Materials and methods. Interscapular brown adipose tissue was taken from hibernating adult male bats (*Myotis lucifugus*). The bats for each individual experiment were obtained not more than 3 days prior to experiment from various caves in Pendelton Co., West Va. In the laboratory the bats were kept torpid (50°F) until their sacrifice by ether. During this study 3 separate preparations were analyzed for androgenic activity. In a typical isolation, the interscapular brown fat from 30 bats was removed, weighed (4.39 g) and saponified with 50 ml of 20% KOH in 85% ethanol under a stream of nitrogen for 2 hours. The nonsaponifiable fraction was dissolved in diethyl ether, thoroughly washed with distilled water and the mixture taken to dryness, *in vacuo*. The nonsaponifiable residue (*Ca.* 86.0 mg) was dissolved in 8 ml of 20% Tween-80 (polyoxyethylene sorbitan monooleate, Atlas Powder Co.). In one experiment, the nonsaponifiable fraction from 4.94 g of liver (21 bats), 34 mg of adrenal glands (30 bats) and 803 mg of testis plus epididymus (30 bats) was prepared as described above.

Biological assays. Response of the seminal vesicles of weanling male rats (avg initial wt., 52.5 g) of the Holtzman Strain to the NFBF from bats was compared to that resulting from various levels of testosterone (Merrell).

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TABLE I. Effect of Testosterone and NFBF on Seminal Vesicles of Intact Weanling Male Rats.

Group*	Mean relative sem. vesicle wt (mg of tissue/g body wt) Exp. I	Exp. II	Exp. III
Tween-80	.30 ±.02†	.24 ±.01†	.23 ±.01†
50 μg testosterone	.38 ±.02	.37 ±.02	.38 ±.03
150 " "	.42 ±.02	.49 ±.03	.64 ±.01
300 " "	.53 ±.01	.59 ±.02	.81 ±.02
NFBF	.48 ±.03	.44 ±.02	.63 ±.02

* Each group in the assay contained 10-11 weanling rats except the NFBF groups which consisted of 3, 3 and 6 animals for Exp. I, II and III respectively.

† Stand. error of mean, $\sigma_M = \frac{\sigma}{\sqrt{N-1}}$.

Animals were divided into 5 groups according to the inoculum received (Table I) and all animals for each experiment were injected concurrently. Daily subcutaneous injections of 0.25 ml were administered on the dorsum of the trunk for 4 days. Testosterone (50 μg, 150 μg and 300 μg standard doses), NFBF and nonsaponifiable matter from liver, adrenal glands and testis plus epididymus were administered in 20%-Tween-80. The control animals received only the solvent. Animals were sacrificed on the fifth day, and seminal vesicles removed and weighed on a torsion balance to the nearest tenth of a milligram. Seminal vesicles were not freed of their contents before weighing. Sample tissues from the seminal vesicles of all animals were fixed in Zenker's fluid, embedded in paraffin, sectioned at 5 μ and stained with Harris's hematoxylin and eosin for microscopic examination.

Results. The response of the seminal vesicle glands of weanling rats to standard doses of testosterone and NFBF for each experiment are presented in Table I. In each experiment NFBF elicited an increase in mean weight of the seminal vesicles of weanling rats equivalent to that produced by 4 daily doses of 150 μg of testosterone. On the basis of mean weight of the seminal vesicles, androgenic activity of the total NFBF (average of experiments I, II and III) may be calculated to be the equivalent of 676 μg testosterone per g. This is a remarkable amount of androgenic activity and vastly exceeds that of bull testis, the richest tissue source heretofore known(4). Control tissue preparations revealed the absence of androgenic material from the liver and small but insignificant quantities of androgenic activity from the adrenal and testis plus epididymus tissues.

Histological change of the epithelium of the seminal vesicles is much less dramatic and allows at best only a subjective evaluation of the androgenic activity of the NFBF. Epithelium of the seminal vesicles of all groups varied from low cuboidal to tall columnar. Nuclei were often basal and degree of granulation of the supranuclear zone of cytoplasm appeared to be in direct proportion to amount of androgen received. Control animals exhibited a greater percentage of low columnar or cuboidal epithelial cells and their supranuclear cytoplasm was often agranular; whereas, the epithelium of animals receiving 4 daily doses of 300 μg of testosterone each was more often tall columnar with the supranuclear cytoplasm frequently granular. The remaining groups (given NFBF and smaller doses of testosterone) demonstrated intermediate states (Fig. 1) in epithelial activity. The stroma was nearly alike in all groups.

Discussion. Information available on the presence of steroids and sterols in the brown adipose tissue of mammals is scarce and contradictory. Clément(5), employing biochemical methods, noted only faint traces of sterols in the interscapular brown fat of rats. Cramer(6) reported positive results in Liebermann-Burchard tests for sterols in warm chloroform suspensions of perirenal brown fat of rats and Tonitti(7) found considerable cholesterol in the interscapular fat bodies of mice. On the other hand Baker, Ingle and Li(8) and Fawcett(9), using the Schultz reaction for cholesterol, were unable to detect sterols in the brown adipose tissue of rats and the Liebermann-Burchard reaction on chloroform extracts of rat brown fat was likewise negative. Fawcett(9) found that birefringence tests for steroids on frozen sections of rat brown fat gave negative results; however, Baker, *et al.*(8) observed abundant birefringent material which they felt suggested the presence of cholesterol or its esters. Fawcett

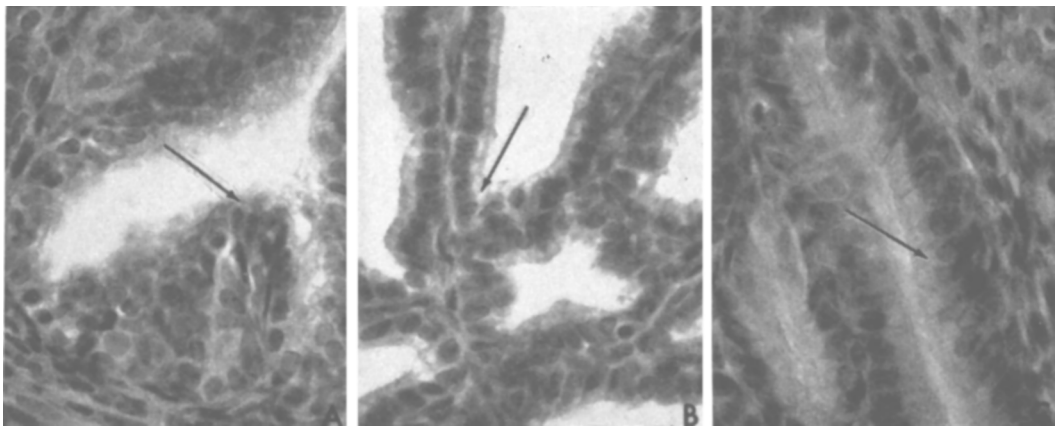

FIG. 1. Photomicrographs of a representative area from the seminal vesicle of: A. Control, B. NFBF and C. testosterone (300 μg/day). Note the difference in amount of supranuclear cytoplasm (at tip of arrow). × 366.

(9) found that the Ashbel and Seligman reaction, which is capable of revealing ketonic steroids *in vitro* and in frozen sections of endocrine glands, gave a negative reaction when applied to brown fat.

As pointed out by Remillard(10) most results seem to indicate the absence of steroid in the brown fat tissues of non-hibernating mammals whereas the few observations available on the brown fat tissue of hibernating mammals suggest that the contrary condition exists.

Remillard(10), using chemical technics, observed small amounts of total cholesterol in the interscapular brown adipose tissue of the female bat (*Myotis lucifugus*). The greatest concentration of total cholesterol occurs in the summer months (highest in August) and minimum concentration is reached in December. Fluctuation in cholesterol levels during summer months seem to accompany drops or rises in water content of the brown fat. Early in hibernation there seems to be a decrease in concentration of total cholesterol, followed by a rise in total cholesterol level during the second half of the period of hibernation. It has been suggested that this later increase may correspond to a preparation for awakening which is accompanied by a higher rate of metabolism and an intensified sexual activity(10). Using histochemical technics, Remillard(10) was able to demonstrate only esters of sterol (principally cholesterol esters).

The high level of androgen in the interscapular brown fat of the bat reported here suggests that the interscapular brown adipose tissue supports accessory gland hypertrophy during testicular involution. Studies are in progress to determine the chemical identity of this androgenic substance† and to ascertain whether or not it is actually synthesized or merely stored in the interscapular gland. Further, it will be of interest to evaluate androgenic potency of the brown fat of the bat during the season of testicular hypertrophy.

Summary. *Myotis lucifugus*, simultaneously exhibits enlarged accessory sex glands and atrophied testis during hibernation. Evidence is presented for presence of an unidentified androgenic substance in the interscapular brown fat obtained from the bat during the hibernation season. The active substance isolated from the nonsaponifiable matter of one gram of brown fat elicits a growth response (rat seminal vesicle assay) equivalent to that produced by 676 μg of testosterone. The presence of an androgenic factor in

† Preliminary gas chromatography of the NFBF revealed the presence of a compound that possesses the retention time characteristic of C-19 steroids. The retention time, however, is unlike that of any steroid previously reported(11), nor is it identical to that of testosterone or androsterone which were analyzed as reference compounds. We are indebted for these analyses to Dr. Charles C. Sweeley, Dept. of Biochemistry and Nutrition, Graduate School of Public Health.

brown fat suggests a unique role for this tissue in the sexual cycle of the bat.

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Effect of Histamine Liberators on Urinary Excretion of 5-Hydroxyindolacetic Acid.*† (26183)

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Bhattacharya and Lewis showed(1) that, in addition to histamine, serotonin is released from rat tissues perfused with Compound 48/80, a potent histamine liberator. Parratt & West(2,3) demonstrated that the anaphylactoid reaction induced in rats by dextran or egg white is chiefly mediated by release of serotonin. Further to these observations, a study was undertaken to evaluate urinary excretion of 5 - hydroxyindolacetic acid (5-HIAA) in rats receiving various histamine liberators. The results reported here show that the output of the serotonin metabolite is unchanged in animals treated with histamine liberators, even after repeated administration. When rats were made resistant to anaphylactoid edema by prolonged treatment with Compound 48/80, they still excreted as much 5-HIAA after injection of reserpine as did the untreated controls.

Materials and methods. Female rats of the Holtzman strain, weighing between 120 and 150 g, were used in these experiments. Twenty-four-hour urine specimens were collected in metabolic cages. During that time, animals were not fed but were adequately hydrated by gastric instillation of 2 ml of tap water 4 times daily. To keep urinary pH sufficiently low, a small amount of acetic acid was added to the flasks. Average urinary output was 10 ml and half of this volume was used for determination of 5-HIAA, following the method of Pierce(4).

In the first experiment, histamine liberators were injected once, at doses indicated in Table I, immediately before animals were caged. *In the second experiment,* Compound 48/80 was administered during 8 days in increasing doses ranging from 200 γ to 1 mg, as reported elsewhere(5), in order to desensitize animals to anaphylactoid edema. In fact, these rats manifested resistance to dextran (6 mg i.p.) and polymyxin B (1 mg s.c.). On the ninth day, they were challenged with 0.5 mg of reserpine, subcutaneously, and placed in metabolic cages for collection of urine.

Results. The values for urinary 5-HIAA are expressed in the Tables as μg/ml. Esti-

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