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Androgen in the Woodchuck Hibernating Gland.

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During the summer of 1939 it was possible for one of us to collect the perfectly fresh tissue from a number of woodchucks *(Marmota monax)*.‡ The tissues obtained immediately after the animals' death were placed in suitable fixing solution for histological study, while the so-called hibernating glands were chopped with scissors into small bits and placed in absolute alcohol containing 1.5% hydrochloric acid. The hibernating gland present in many hibernating and a few non-hibernating mammals has attracted the attention of a number of investigators but has yielded remarkably little information as to its function. The distinct histological character of the glandular adipose tissue as compared with ordinary fat seems to be established.[1] In the woodchuck three-fourths of the body fat is lost during hibernation while the hibernating gland is not utilized to any extent during this period. During the 3 to 4 weeks following emergence from hibernation, which is said to be the mating season, the hibernating gland shrinks rapidly to one-fourth of its original weight, while all but a trace of the body fat is lost. The manner in which this tissue is spared during the hibernating period and utilized during the breeding season has not been explained.

During the course of these dissections an impression was gained that some material giving rise to the brownish color of the hibernating glands was being deposited in pre-existing fat lobules, rather than that the hibernating gland is a tissue entirely distinct from the neighboring fat.

In one old male animal the testes presented a striking, almost black, color. There appeared to be some of this same pigmented material around the urethra and also in the adrenals.

The question arose in our minds as to whether some pigmented substance was being elaborated by the interstitial cells of the testis and being stored in the hibernating glands; it is known that in the true

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‡ We are greatly indebted to Dr. and Mrs. George H. Kittell, of Jamaica, L. I., expert riflemen, for this opportunity.

1 Rasmussen, A. T., *J. Morph.*, 1923, **38**, 147.

hibernating animals the interstitial cells are apparently very active during the summer, while the spermatogenetic tissue seems to have almost disappeared. This idea occurred to us after the tissue had been brought back to the laboratory, and we realized that we did not have adequate controls for anything other than a preliminary experiment. However, we felt justified in trying a bioassay of the material for androgenic content, and the result is so striking that we feel free to present a preliminary communication, again emphasizing our appreciation of the incomplete character of the study.

The available tissue represented some 12 animals, both males and females. Unfortunately the glands were not kept separate as to sex. Eight months after collection the alcohol was poured off, the glands weighed (53 g), hashed, and extracted repeatedly with 95% alcohol on the steam bath. The tissue residue was then extracted for two 6-hour periods with ether in a Soxhlet extractor. The combined alcohol-ether extracts, including the original acid-alcohol solution, were evaporated and the residue dissolved in acetone, the solution chilled to remove phospholipins and the acetone evaporated. The residue was distributed between 70% alcohol and petroleum ether and both fractions evaporated. The weight of the petroleum ether fraction was 22.0 g, the 70% alcohol fraction 2.5 g. Both were assayed for androgen on the capon comb by the inunction method in parallel with a solution of pure androsterone as a standard. The petroleum ether fraction was inert. The alcohol fraction contained the equivalent of 110 μg (0.110 mg) androsterone in the 53 g of tissue. This is equal to 1 I.U. (International Unit) of androgen (100 μg of androsterone) in 50 g of tissue. Bull testis, the richest tissue source of androgen yet reported, contains 1 I.U. in 50-150 g.[2] Due to dehydration by the alcohol the fresh weight of the hibernating glands was doubtless appreciably greater than the 53 g noted but the amount of androgen found places this tissue within the range of the findings in bull testis.

It is impossible to evaluate this finding until other tissues of the woodchuck can be assayed for androgen. Its presence or absence in the body fat would be of special interest. Since we were unable to obtain more woodchuck material we investigated the androgen content of human male and female axillary fat.§ Although small amounts of androgen were found, equivalent to 1 I.U. in 1 kg of

2 Koch, F. C., *Physiol. Rev.*, 1937, **17**, 153.

§ We are grateful to Dr. Jacob Furth, Department of Pathology, The New York Hospital, and to Dr. Jacob Werne, St. John's Hospital, Queens, N. Y., Assistant Medical Examiner of the Borough of Queens, for securing this material.

male (age 46) and 1 I.U. in 3 kg of female (age 55) axillary fat, these concentrations are so low as to be of questionable significance. (The age of the subjects conceivably may have been responsible for the low androgen content.) This marked difference in androgen concentration between the woodchuck hibernating gland and human axillary fat suggests that a high androgen content is not a property of all fatty tissues but may reflect the part played by the hibernating gland in the sex cycle of the woodchuck.

Any theorizing as to the significance of the androgen in the woodchuck glandular adipose tissue must await confirmation of this finding and a study of its concentration in other tissues of the animal. In view of the functions already attributed to the hibernating gland: a protein-sparer;[3] a storage point for vitamins and lipoids;[4] and the source of a metabolism-depressing secretion,[5] we hesitate to attach any functional significance to the presence of androgen in the gland until further evidence is available.

Summary. The hibernating glands of woodchucks killed during the summer contained 1 International Unit of androgen, (100 μg androsterone) in 50 g of tissue, equal to the concentration in bull testis, the richest tissue source known. Human male and female axillary fat contained 1 International Unit in 1 kg and 3 kg respectively.

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A Laboratory Method for the Soilless Growth of Grass.

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Fresh green plant material is frequently used to supplement standard stock diets in the raising of laboratory animals such as the rat. Recently, additional impetus has been given to the study of young green growing plants by observations on the previously unknown factor (factor *pl* of Wulzen and Bahrs,[1] grass juice factor of

3 Vignes, H., *C. R. Soc. Biol.*, 1913, **75**, II, 360, 397, 418.

4 Cramer, W., *Brit. J. Exp. Path.*, 1920, **1**, 184.

5 Wendt, C. F., *Z. physiol. Chem.*, 1937, **249**, 2.

1 Wulzen, R., and Bahrs, A. M., *Physiol. Zool.*, 1936, **9**, 508.