Metabolic and Biochemical Changes in Testis of Cotton Rat (*Sigmodon hispidus*) During Breeding Cycle.* (30005)

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Testis and accessory organs of seasonal breeding males undergo remarkable changes during one year of life. Mossman *et al*(1) reported that degenerating genital organs of seasonal breeding males compare morphologically to the senescent organs of other mammals. These degenerated gonads soon change from a senile condition to an embryonic state and proceed from it to full function again. Several investigators(2-6) have shown that morphological, histological and weight changes occur in testes and accessory organs of several species during one breeding cycle. Wislocki(7) investigated the phenomenon of seasonal breeding activity using histochemical techniques. He observed seasonal changes in the testis, epididymis and seminal vesicles of the Virginia deer (*Odocoileus virginianus borealis*). These changes encompassed lipids, steroids, glycogen and acid and alkaline phosphatases. The study presented herein carries these investigations one step further and measures metabolic and biochemical changes in testis of a seasonal breeding mammal (*Sigmodon hispidus*) during the breeding and non-breeding seasons.

Materials and methods. During a one-year period, June 1, 1963-May 30, 1964, 91 male cotton rats were collected from their natural habitat in 2 trapping areas. Animals were brought into the laboratory from the field every month (with exception of July and August) in live traps in which they were caught. They were sacrificed by cervical dislocation after which testes were excised, chilled to 4°C and weighed. Accessory organs were removed and weighed also. One testis was used to prepare teased tubules for manometric studies as described by Umbreit *et al*(8). Oxygen consumption was measured manometrically by the direct method of Warburg(8). The gas phase was air, and incubation period 2 hours. The incubation medium was either glucose-free Locke's solution or Locke's solution with .055 *M* glucose as the exogenous substrate. Aliquots were taken initially and after incubation and analyzed for glucose(9) and lactic acid(10). A 20% (w/v) double distilled water homogenate was prepared from the remaining testis. An aliquot of this homogenate was assayed for protein according to Lowry *et al*(11). The remainder of the homogenate was used for extraction of nucleic acids(12) and assayed for ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) according to Ceriotti (13) and Burton(14), respectively. The data were grouped into 3 time periods as follows: 1) Active breeding season (June-September), 2) Period of degeneration (October-January) and 3) Period of redevelopment (February-May). This information is presented as mean values ± standard error of mean.

Results. It is apparent that testis weight was much lower during the periods of degeneration and redevelopment than during active breeding season (Table I). These changes in testis weight were concomitant with a decrease in accessory organ size.

Table II shows the change in oxygen uptake, glucose uptake and lactic acid production in testis from these animals during the 3 seasonal periods. Endogenous oxygen uptake was lowest during the periods of active breeding season and degeneration and reached a peak during the period of redevelopment. Oxygen uptake in the presence of exogenous substrate was greatly stimulated during the active breeding season, reduced during the period of degeneration and intermediate during redevelopment. Glucose uptake did not change appreciably during the entire year. Lactic acid production was inversely related to oxygen uptake. During the period of de-

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TABLE I. Effect of Season on Testis, Seminal Vesicle and Prostate Gland Weights.

	Period of redevelopment	Active breeding season	Period of degeneration
	Feb-May	June-Sept	Oct-Jan
Testis wt (mg)	569 ± 134 (n = 25)	2268 ± 94 (n = 25)	750 ± 155 (n = 41)
Seminal vesicles (mg/100 g body wt)	8.7 ± 2.5 (n = 20)	979 ± 106 (n = 15)	95 ± 31 (n = 40)
Ventral prostate (mg/100 g body wt)	0 (n = 20)*	219 ± 32 (n = 15)	28.5 ± 9 (n = 40)

* During period of redevelopment the ventral prostate was so small that it could not be found and dissected out.

TABLE II. Effect of Season on Oxygen Uptake, Glucose Uptake and Lactic Acid Production of Testis.

	Period of redevelopment	Active breeding season	Period of degeneration
	Feb-May	June-Sept	Oct-Jan
Oxygen uptake (μl/mg dry wt/hr)			
Endogenous*	8.22 ± .65	6.28 ± .37	6.66 ± .36
Substrate added†	7.53 ± .42 (n = 19)	8.96 ± .45 (n = 23)	6.12 ± .39 (n = 32)
Glucose uptake (μg/mg dry wt/hr)	90.16 ± 11.70 (n = 19)	80.08 ± 5.39 (n = 24)	95.41 ± 10.45 (n = 30)
Lactic acid production (μg/mg dry wt/hr)			
Endogenous*	1.66 ± .99	1.77 ± .50	6.62 ± 1.89
Substrate added†	17.78 ± 2.78 (n = 19)	26.55 ± 2.07 (n = 25)	43.92 ± 5.95 (n = 25)

* Incubation medium was glucose free Locke's solution.
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TABLE III. Effect of Season on Protein, Ribonucleic Acid (RNA) and Deoxyribonucleic Acid (DNA) Content of Testis.

	Period of redevelopment	Active breeding season	Period of degeneration
	Feb-May	June-Sept	Oct-Jan
Protein content (mg/g testis)	83.21 ± 3.86 (n = 22)	76.17 ± 1.63 (n = 22)	74.81 ± 2.66 (n = 22)
RNA content (mg/g testis)	5.55 ± .19 (n = 19)	3.08 ± .12 (n = 25)	2.95 ± .16 (n = 23)
DNA content (mg/g testis)	7.82 ± .74 (n = 19)	6.37 ± .26 (n = 24)	6.78 ± .47 (n = 22)
RNA/DNA	.710	.484	.435

generation, when oxygen uptake was at its minimum, lactic acid production was at its highest point.

Table III shows total protein, RNA and DNA content of testicular tissue of these cotton rats. Protein content appeared to reach a maximum during the period of redevelopment. It is apparent that RNA and DNA were greatly elevated during this time period.

Discussion. These data indicate that male cotton rats reach a peak of reproductive potential during one period of the year. During this time the testis and accessory organs reach their maximum size. Green(15) studying female cotton rats from the same population has shown that this correlates with a

peak in female reproductive capacity as measured by number of females pregnant, with vulva open and the number lactating. It seems logical that these variations in reproductive function must be accompanied by metabolic and biochemical changes which if understood could provide insight into the regulation of circadian cyclic phenomena. Oxygen uptake, and lactic acid production indicate that during the period of degeneration, testes shift from a metabolism characterized by a high oxygen utilizing system to one in which glycolysis predominates. The fact that glucose uptake remains virtually unchanged suggests that one pathway is operating at the expense of the other. This trend is reversed during the period of redevelopment and it is interesting to note that endogenous oxygen uptake reaches a maximal point at this time. This observation is similar to that found in immature rat testes by Tepperman and Tepperman(16). Since testes increase approximately 4 times in size during the period of redevelopment, one would expect an increase in protein synthesis. This hypothesis is supported by the fact that RNA and protein content of testis and that the RNA/DNA ratio is greatly increased during this time. Deoxyribonucleic acid content also is increased and probably reflects an increase in cell number. Therefore these data indicate that cyclic breeding activity of the male cotton rat is correlated with profound physiological and biochemical changes in the testis of this seasonal breeding mammal.

Summary. A total of 91 cotton rats (*Sigmodon hispidus*) were collected from their natural habitat once a month for one year. Testes and accessory organs were excised and weighed. Teased tubule preparations of the testis were used to measure oxygen uptake, glucose uptake and lactic acid production. The remainder of testis was assayed for protein, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). These data indicate that characteristic metabolic and biochemical changes take place in testis during circadian breeding patterns.

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Response of Intraocular Ovarian Isografts to LH in Male Mice.* (30006)

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Ovariectomized mice, bearing bilateral intraocular ovarian isografts, show normal vaginal cycles. The grafts repeatedly develop mature follicles and corpora lutea in relation to proestrus-estrus and metestrus-diestrus, respectively(1). Identical grafts in *intact* males develop mature follicles which, while they

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