Chronic maintenance of rat sperm reserves into old age by previous sexual contact

G. T. Taylor*, J. Weiss** and J. Royalty***

*Tokyo University-Komaba, Department of Biology, Tokyo 183 (Japan), **Universität Heidelberg, Zentrum für Molekular Biologie, D-6900 Heidelberg (Federal Republic of Germany), and ***Murray State University, Murray (Kentucky 42071, USA), 14 May 1986*

Summary. Extensive copulatory experiences at a younger age provoked chronic changes in the reproductive physiology of old male rats. Continuous access to females was not necessary, sperm numbers were elevated in the 22-month-old males 7 months after a final sexual interaction.
Key words. Copulation; sperm; aging; rats.

Reproductive senescence in male mammals is a process that begins in middle age, for example, sperm quantity and circulating levels of testosterone are gradually reduced in the aging male[1]. Nevertheless, there are sizeable individual differences in the rate and nadir of the decline[2]. Reproductive functioning among young adult male conspecifics, too, is highly variable[3] due to a host of ontogenetic-endocrine-environmental factors. One manipulation to increase activity in the reproductive system is to expose the younger male to a sexually receptive female. Copulatory experience is followed by marked changes in sexual responding[4], secretory activities of sex accessory glands[5] and morphology of penis and penile papillae[6].

Many of these changes in the androgen-sensitive reproductive system are long-lasting and resist, more or less, reductions in circulating levels of testicular hormones[7]. We have proposed a hypothesis[8] that acute endogenous pulses of hormones, such as the pulse of testosterone that accompanies copulation, provokes chronic structural changes. Minimal levels of the hormone, subsequently, can maintain proper functioning of the tissue. We report here on chronic influences on testicular and epididymal sperm of aged rats with extensive previous sexual experience.

The 44 male Wistar rats were 3 months old at the beginning of the experiment and were sacrificed at 22 months of age. Each male was housed for one year, ending when each male reached 15 months of age, either with two gonadally intact or with two ovariectomized females. There were 22 males in each group. Females were changed at random intervals to ensure that the males received varied experiences with females; pregnancies were monitored to confirm the multiple sexual experience. Before and after the year with females, the males were housed three per cage.

Sperm were counted using a procedure described in previous papers[1,6]. Briefly, epididymides were excised, placed in a glass dish with 2 ml 0.9% (w/v) NaCl and gently minced with a razor blade. Testes were removed, weighed and homogenized in 2 ml NaCl. A single ml of the homogenate was mixed with 1 ml 1% Triton C-100 solution and treated for 1 min with an ultrasonic disentegrator at 35 W. That suspension was diluted 1:3 with distilled water and 1 ml diluted suspension was added to 1 ml 1.54 M acetic acid. The solution was mixed by vortexing, and a drop of the suspension was placed in a hemocytometer.

Sperm were counted from 7 chambers of the hemocytometer; the extreme scores were eliminated and a mean was obtained from the middle 5 values. Two samples were counted from each side of both organs. Separate samples were used to evaluate the percentage of motile spermatozoa from the epididymides. The findings for the two groups of males are reported in the table as means ± SEM.

The data were analyzed statistically with t-tests. Results for organ weights revealed a statistically significant difference for epididymides ($t(42) = 4.89$, $p < 0.01$), but not testes ($t(42) < 1$). Similarly, differences in testicular sperm counts expressed as sperm/g tissue did not achieve statistical significance ($t(42) = 1.82$, $p < 0.10$), yet epididymal sperm counts per g tissue were significantly different ($t(42) = 6.69$, $p < 0.01$). Because total numbers of sperm may be an important predictor of fecundity[9], testes and epididymides were analyzed for total sperm counts. Differences in obtained values for both organs achieved statistical significance ($t(42) = 2.13$, $p < 0.05$, and 11.45, $p < 0.01$, respectively). Analyses of percentages of motile spermatozoa from epididymides indicated that the differences between the groups were not statistically significant ($t(42) = 1.82$, $p < 0.10$). To summarize, results indicate that copulation elicits changes that are observed chronically in both epididymides and testes. Epididymides responded with an elevation of weights and sperm reserves, although apparent increased spermatozoan motility failed to achieve statistical significance. The response of testes to prior sexual contact was more equivocal, for only the total numbers of sperm were elevated above the sperm number for virgin males.

Sperm analyses of 22-month-old rats that were either virgins or had received extensive sexual experiences seven months prior to the analyses

Measure	Experienced (n = 22)	Inexperienced (n = 22)
Testes		
Weight (g)	3.674 ± 0.100	3.650 ± 0.057
× 10^6/g tissue	75.20 ± 2.25	69.16 ± 2.24
Total (× 10^6)	274.64 ± 9.66*	250.68 ± 5.08
Epididymides		
Weight (g)	1.121 ± 0.20**	0.975 ± 0.017
× 10^6/g tissue	394.23 ± 16.66**	257.55 ± 9.94
Total (× 10^6)	431.86 ± 10.71**	251.82 ± 11.12
Motility (%)	38.84 ± 0.99	33.00 ± 1.40

*Statistically significant differences, t-test $p < 0.05$ (*) or $p < 0.01$ (**).

The findings are consistent with previous reports that continuous cohabitation with females slows reproductive senescence[10], although sexually experienced and inexperienced male mice may be indistinguishable at very old age[11]. Data from the present study support the hypothesis that acute exposure to environmental agents chronically modifies reproductive functioning.

A plausible explanation for the differences between sexually experienced and inexperienced males is a different hormonal profile. Exposure to a receptive female releases acute pulses of gonadotrophic and testicular hormones[12]. Our findings suggest that the hormonal pulses effect changes in epididymal sperm reserves and, to a lesser extent, testicular spermatogenesis. It is notable that these were chronic changes[13]. Specifically, copulatory experience during earlier stages of the adult ontogeny of male rats was capable of maintaining increased numbers of sperm into old age, although females were removed 7 months previously, or 35% of the lifespan of a rat[1].

A testable hypothesis in future research is that behavior provokes basic structural modifications in hormone-sensitive tissue that is characterized by increased affinity for the existing levels of circulating hormones. The consequence would be that the reduced testosterone with aging[14] is less disruptive to various aspects of the reproductive system in experienced males. Sperm production and storage may be one example[15].

*Present address: Department of Psychology, Laboratory for Psychobiology, University of Missouri-St. Louis, 8001 Natural Bridge Road, St. Louis, MO 63121 (USA).

Acknowledgments. The research was supported, in part, by grants from the University of Missouri (USA), Alexander Von Humboldt Foundation (FRG) and Japan Society for the Promotion of Science.

1 Taylor, G.T., Weiss, J., and Frechmann, T., J. Reprod. Fert. *77* (1986) 419.
2 Finch, C.E., Jones, V., Wisner, J.R., Sinha, Y.M., Devellis, J.S., and Swerdloff, R.S., Endocrinology *101* (1977) 1310.
3 Harding, C.F., and Feder, H.H., Brain Res. *105* (1976) 137.
4 Dewbury, D.A., Anim. Behav. *17* (1969) 217.
5 Agmo, A., Physiol. Behav. *13* (1974) 41.
6 Taylor, G.T., Weiss, J., and Komitowski, D., J. Endocr. *98* (1983) 155.
7 Dahlof, L.G., and Larsson, K., Behav. Biol. *24* (1978) 492.
8 Taylor, G.T., Weiss, J., and Haller, J., Anat. Rec. *211* (1985) 304.
9 Schwartz, D., MacDonald, P.D.M., and Heuchel, V., Reprod. Nutr. Devl. *21* (1981) 979.
10 Drori, D., and Folman, Y.J., J. Reprod. Fert. *8* (1964) 351.
11 Bronson, F.H., and Desjardins, C.J., Gerontology *37* (1982) 555.
12 Kamel, F., and Frankel, A.I., Endocrinology *103* (1978) 2172.
13 Harding, C.F., Am. Zool. *21* (1981) 223.
14 Konoshita, Y., Higashi, Y., Winters, S.J., Oshima, H., and Troen, P., Biol. Reprod. *32* (1985) 309.
15 Taylor, G.T., Weiss, J., Frechmann, T., and Haller, J., J. Reprod. Fert. *73* (1985) 323.

0014-4754/87/0311-02$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1987

Changes in binding of JH-III in hemolymph of adult female *Locusta migratoria*

F. Couillaud*, A.B. Koopmanschap, C.A.D. De Kort and A. Girardie**

*Laboratory of Entomology, Agricultural University, P.O.Box 8031, NL-EH Wageningen (The Netherlands), **Laboratoire de Neuroendocrinologie, UA CNRS 1138, Université de Bordeaux I, Avenue des Facultés, F-33405 Talence Cedex (France), 2 June 1986*

Summary. Hemolymph from adult female *Locusta migratoria migratorioides* was analyzed for binding of juvenile hormone III (JH-III) after allatectomy and transection of the *nervus corporis allati* 1 (NCA-I). These operations did not affect the apparent dissociation constant of the binding (Kd = 3.3 10^{-8} M). The concentration of binding sites exhibited fluctuations in relation to age and type of operation: an increased concentration of binding sites in females with disconnected corpora allata and a decreased concentration in allatectomized females. The changes in concentration of binding sites was not due to differences in water content or hemolymph volume in operated animals. The hemolymph protein concentration was reduced after NCA-I transection and even more after allatectomy. However, variations in protein concentration did not correlate with changes in concentration of JH-III binding sites. The changes in binding site concentration were related to changes in JH-titer.
Key words. locust; corpora allata; JHBP; hemolymph protein content; hemolymph volume.

In insect hemolymph, juvenile hormone (JH) has been shown to associate specifically with binding proteins (JHBP)[1,2]. The JHBP facilitate JH transport from the corpora allata to the target tissues and prevent degradation by carboxyesterases or unspecific binding to the body wall and tissue membranes. Furthermore, it has been suggested that JHBP may be involved in recognition of the target cell[3,4].

On the basis of affinity, specificity and binding capacity, 2 classes of JHBP have been identified in Lepidoptera: low affinity high molecular weight lipoprotein and high affinity low molecular weight protein[1]. Moreover, in insects like *Locusta migratoria* with only JH-III (C-16) as natural hormone[5], hemolymph contains a third class of JHBP with high affinity and high molecular weight[6].

By protecting JH against carboxysterases, the concentration of JHBP may have a significant influence on the JH titer. In *Manduca sexta* larvae, direct correlation between relative hemolymph JH titer and the level of JHPB was noted only at the beginning of the fourth and the fifth instars. During the rest of the fourth instar JH and JHBP concentrations appeared to be inversely related[2]. In the present study, fluctuations in JHBP concentrations were investigated in batches of female *Locusta migratoria* known to contain different JH titers: 1) adult females with increasing JH titers during oocyte maturation[7], 2) allatectomized females without JH after removal of the JH source[7] and 3) females with corpora allata disconnected from the *nervus corpori allati* I (NCA-I). NCA-I transection results in higher JH titers than in controls, despite low rates of JH biosynthesis by these corpora allata[7].

Allatectomy and NCA I transection were performed on day 1 adult females as previously described[8]. Hemolymph samples were collected into capillary pipettes after cutting the neck membrane. Hemocytes were removed by centrifugation (10,000 g, 5 min, 4 °C) and hemolymph from 5 animals was pooled.

Native gradient PAGE (slab gels 4–20%, c = 2.7%, 2 mm thick, used horizontally with 0.11 glycine titrated with Tris pH 8.9, 24 h with constant 200 V at 10 °C) of hemolymph from controls, allatectomized and disconnected corpora allata females, revealed qualitatively similar patterns (results not shown). According to de Bruyn et al.[9], a band with molecular weight of 575,000 Dalton can be identified as JHBP. Lower staining intensity was observed for the JHBP band in hemolymph from allatectomized females, but the total protein content was also lower[10].

To investigate changes in JHBP content of different hemolymph samples, we used Scatchard plot analysis. Increasing amounts of JH-III (Calbiochem) were incubated with a constant amount of hemolymph and the hormone-protein complex precipitated with polyethylene glycol (PEG)[11]. The concentration of JH-III in ethanolic solution was checked spectroscopically at 220 nm (E = 13,800)[12]. Carboxyesterase and protease activities were inhibited by Para-oxon (10^{-4} M) and PMSF (Phenyl methyl sulfonyl fluoride 10^{-4} M) respectively. JHBP of *Locusta* bind efficiently and specifically to 10R JH-III[6,13]. A very small amount of hemolymph (1 µl in 800 µl TMK buffer) was used to bind about 50% of racemic [10 ^{3}H] JH-III (NEN Corporation) at the concentration of 10^{-9} M.

Scatchard plot analysis of [10 ^{3}H] JH-III binding to hemolymph

Water content (determined by the ratio (fresh weight − dry weight)/fresh weight) and hemolymph volume (determined by labeled inulin dilution) in day-1 allactectomized females, day-1 NCA-1 transected females and control females. Means ± SEM; n, number of individual determinations

Females	Water content (%)	Hemolymph volume (µl)
Control	70.07 ± 0.68 (11)	794.73 ± 72.00 (11)
NCA-transected	71.85 ± 1.07 (12)	915.25 ± 67.64 (12)
Allatectomized	69.93 ± 1.08 (13)	774.58 ± 75.03 (12)