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Initial Results of Chemical Inhibitors and Photoperiodic Influences on Growth and Reproduction in *Microtus pennsylvanicus*

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Recently Berger *et al*. (1977) have demonstrated reproductive inhibition in *Microtus montanus* as a result of specific plant compounds in natural vegetation. Naturally occurring cinnamic acids and their related vinyl phenols have been demonstrated to have marked effects on uterine weight, inhibition of follicular development and cessation of breeding activity. Compounds having antigonadotrophic and antithryotropic activities have been identified in a wide variety of plants (Chury 1967). Bickoff *et al*. (1959) report that alfalfa contained a non-estrogenic compound which would over ride the estrogenic effect of the plant estrogen coumestrol. Adler (1962) demonstrated that a non-estrogenic compound in alfalfa could inhibit estrogenic responses of natural animal estrogens. Allison and Kitts (1964) extracted yellow pine needles and demonstrated that they contained a factor which would depress the uterine weight of immature weanling mice thereby delaying sexual maturation. Other investigators have reported similar findings but all failed to identity the compounds producing the effect. Gasser *et al*. (1963) isolated an inhibitory substance named lithospermic acid and other investigators have demonstrated it deactivates pituitary gonadotrophins in vitro.

Berger *et al*. (1977) report the effect of four plant compounds on uterine weight, follicle development, and breeding performance on *Microtus montanus*. The plant compounds utilized were extracted from winter wheat sprouted to 10 cm, homogenized and subjected to steam-ether extraction for 7 days. Three fractions extrated were analyzed by gas chromatography and subsequently tested on young *Microtus montanus*. One fraction when biologically assayed demonstrated an inhibition of uterine development. Further chemical extraction of this sample resulted in its characterization by nuclear magnetic resonance as containing a vinyl group and disubstituted phenol. Subsequent testing of 4 vinyl guaiacol by bioassay, gas chromatography and NMR analysis showed it to be identical in its chemical properties and biological activity to the plant compound. The parent compounds in the plant, PCA (Para coumaric acid) and FA (Ferulic acid), are found in low concentrations in young growing vegetation, but increase to 4 mg/gm as a plant reaches senescence. Although both PCA and FA are effective in depressing uterine weight their decarboxylated vinyl phenols (4 VG and 4 VP) are much more effective.

The compounds 4 VG and 4 VP when administered for 7 days on ground lab chow at 1 mg/gm significantly reduced uterine development in young Microtus montanus and hence delayed sexual maturity. The parent compounds (PCA and FA) after 12-14 days of treatment caused a significant reduction of uterine development. Examination of the ovaries of the 4 VG group showed significant reduction in the number of secondary uterine follicles. When tested on breeding adults over a 100 day period the chemical treatment resulted in a reduction of the number of litters born, number of young per litter and in the number of females still breeding at the end of the test period. When 4 VG was tested on male Microtus montanus by intraperitoneal injections at 1 mg/day for 3 days, a significant reduction in serum testosterone occurred. These data are suggestive that 4 VG also affects the male reproductive system in a way similar to its effects on females.

Negus et al. (1977) has demonstrated the existence of a plant compound called "stimulator" (now identified pers. comm. N. C. Negus) which when tested in a previously non-breeding population of M. montanus caused a resumption of breeding activity. They have also demonstrated that following seasonal drought breeding resumes within 3 to 5 days following the resumption of active plant growth. They conclude that the cessation of reproduction in Microtus montanus populations is associated closely with the build up of inhibitory compounds in food plants in the absence of stimulatory compounds. They have demonstrated that in populations where reproduction has ceased, under non-stimulatory photo-periodic conditions, reproduction can be initiated by the addition of the stimulatory compound to the environment.

Cengel et al. (1978) monitored reproduction of Microtus pinetorum in abandoned and maintained orchards. They demonstrated differences in reproduction and related these to quality of food and fat deposition. One of the differences in food types between the two habitats was the abundance of grass which was kept in an active growth stage in the maintained orchard. These results could indicate a possible role of plant stimulatory and plant inhibitory compounds on reproduction in the pine vole. La Voie and Tietjen (1978) indicated that the role of grass in orchard environments and the effects of temperature and photoperiod on activity patterns and reproduction need additional investigations. This laboratory is currently investigating these parameters on both problem voles (M. pennsylvanicus, M. pinetorum).

Methods and Procedures

The data to date on reproductive inhibition in microtine rodents has been limited to maturation of subadults and reproductive potential of adults. This laboratory is currently testing the effect of inhibitory substances on reproduction in M. pennsylvanicus and has initiated testing of plant stimulatory compounds. Animals utilized in laboratory experiments were derived from continously outbred laboratory populations established in 1978 at Virginia Polytechnic Institute and State University. The test chemicals PCA and FA were obtained from Aldrich Chemical Corporation as their structure has been verified to be the same as those extracted from common orchard grass. The stimulator fraction has been produced from an acetone-ether extraction from sprouted oats grown in a sterile medium (TA 24^{o}C, LD 16:8) in order to insure a constant supply and a constant quality.

The bioassay for effects on sexual maturation followed those of Berger-et al., (1977) who determined that wet uterine weight and histological examination of follicle types were the best assay measures of chemical effects on reproduction. Total food consumption, body weight, adrenal weight and total body fat were assayed to determine the chemical effects on the general condition of the test animals.

Animals to be tested are selected at random at 18 days of age and at approximately equal weights, and are caged as individuals for the entire test period (14 or 21 days). The test chemicals are disolved in methanol and then coated on to ground laboratory animal chow, control diets are treated in the same way and the two diets are then air dryed for 24 hours.

The effect of photoperiod on reproductive maturity and growth are currently under experimental testing for both M. pennsylvanicus and M. pinetorum. Growth rates of young M. pennsylvanicus under LD 18:6, LD 6:18, and natural photoperiod were determined by weighing and measuring individuals every 1 to 3 days for up to 10 weeks. Radioimmunoassay for T-4 levels in young raised under LD 18:6 and LD 6:18 were determined using a standard T-4 kit to assess photoperiodic effects on metabolism.

Results and Discussion

The effect of PCA and FA administered at doses of 4, 8, and 12 mg/gm of chow per day for 14 days showed a trend toward a significant difference in uterine weight at the end of the test period. Tests at 12 mg/gm of chow over a 21 day test period gave a highly significant (P= .02) difference in uterine weight. Further tests at 4 and 8 mg/gm of chow over 21 day test periods are currently under way. In all tests there were no significant differences between control and experimental groups in fat deposition, food consumption and body weight. In the 21 day test at 12 mg/gm of chow the adrenals of the experimental groups were larger 12.7 $\pm$ 2.9 mg than the control adrenal weights which were 10.6 $\pm$ 1.9 mg net weight. As these groups were under a photoperiodic regime which stimulates constant reproduction this difference is most probably due to an adrenal response to the chemical inhibition of reproductive activity. Histological examination of the ovaries and adrenals will provide additional data on tissue responses to the test chemicals. Maturation of secondary follicles ceased in the ovaries of M. montanus which results in a cessation of reproduction due to the chemical treatment. When the ovaries of all test groups are examined their overall response to the various test chemicals will produce a clear picture of the effects of the experimental diets in M. pennsylvanicus. The long photoperiod tests were utilized as maximum growth and breeding is achieved under these conditions, though other photoperiod lengths are being studied.

Growth of M. pennsylvanicus under long and short photoperiods (LD 18:6 and LD 6:18) differs significantly. Young raised from birth to weaning on the short photoperiod are 3 grams lighter at weaning. After weaning both long and short photoperiod groups grow at the same rate but the short photoperiod group remains significantly smaller at the end of the 30 day test period. In order to determine the effects of natural photoperiod on growth and development 3 cohorts (15 individuals in each) born in June, July, and September were placed under natural photoperiodic and temperature conditions in October and monitored through January. Individuals born in June and July reached adult size and weight ($\bar{x}$=58gms) by October and lost weight ($\bar{x}$=50gms) until the winter solstice under the effect of the decreasing photoperiod. The September-born cohort gained weight under the decreasing photoperiodic regime reaching the same size and weight as the June and July cohorts at the Winter solstice. All cohorts are now gaining weight under incresing photoperiodic conditions.

Four groups containing 10 subadults each ($\bar{x}$ weight=48 gms) were set up as pairs under photoperiods of LD 18:6 or LD 6:18 for 10 weeks after which one of each pair was switched to the opposite photoperiod. Subadults under LD 18:6 reached adult size and weight ($\bar{x}$=58 gms) within 16 weeks while those under LD 6:18 lost weight ($\bar{x}$=42gms) during this period. At the time of switching the groups there was no significant differences from the control pairs but after 6 weeks on the opposite photoperiod such differences were apparent. M. pennsylvanicus adults and subadults lost weight under the short photoperiodic conditions and gained weight under the long photoperiod. Radioimmunoassay of T4 levels show significantly reduced levels in adult animals under short photoperiodic conditions.

Most of the data developed on M. pennsylvanicus to date characterizes the general response of the animal to its environment. The incorporation of chemical stimulators and inhibitors in this environment alters these general responses to synchronize important events like reproduction, fat deposition and altered metabolism. The aim of this research is twofold: 1) to understand biologically how these compounds affect both problem vole species and 2) to reduce the availability of stimulator compounds while augmenting inhibitor substances to cause a reduction or cessation of reproduction.

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