Some Dietary Effects on the Estrous Cycle of the Female California Pocket Gopher, Thomomys bottae navus Merriam

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Introduction

In recent years the effect of diet on the reproductive cycle of mammals has received increasing attention (1, 5, 11). Pioneering such work have been the studies of Greep (3) and others. Moore (9) reviews some of the dietary factors believed to be responsible for male sterility.

The male pocket gopher in California appears to have two basically different types of reproductive cycles (4). In the vicinity of Davis, California, where the land is irrigated during the dry season, gophers breed during the entire year. In unirrigated land gophers appear to breed on a strictly seasonal basis. While investigating the reproductive activity of male gophers living in irrigated land, Gunther (4) suggested that the influence of a habitat in irrigated fields may be responsible for the drastic shift from a strictly seasonal breeding behavior to a continuous cycle.

If the influence of habitat is responsible for a continuous breeding activity in gophers in irrigated fields, there are several factors or sets of factors within such a habitat which can be profitably investigated. In this paper a preliminary report on the effect of diet as a possible factor responsible for the continuous breeding activities of the female gopher will be considered.

Materials and Methods

The observations reported here were made from studies on a total of nine freshly captured female gophers. All animals were live-trapped in irrigated alfalfa fields on the University of California Farm at Davis, California, and were processed in the laboratories of the Department of Zoology, University of California, Davis, California.

Upon capture the animals were weighed, vaginal smears were taken and then the females were placed singly in solid-walled aluminum cages open at the top. Dirt to a depth of eight inches was placed in each cage, and was moistened from time to time. A metal trough was placed on top of the dirt, affording a covered, surface runway for the animals.

Weighing of the animals at time of capture and during the course of the experiment was deemed desirable in order to check against factors which might cause loss of weight and thus obviate the results of the experiment. Furthermore, since nothing was known of the age of these animals, it was necessary to establish an arbitrary weight limit of 90 grams or over to designate sexually mature females. This limit was set since only a small number of pregnant females weighing less than

90 grams had been captured, while animals over this limit were consistently pregnant.

In order to determine whether an easily recognized estrous cycle could be induced in the pocket gopher, comparable to that identified in the rat and guinea pig (7), three animals were given daily subcutaneous injections for one week of 100 RU of estradiol benzoate (Progynon-B, Schering Corporation). Two other females were injected daily for one week with 100 RU of estradiol benzoate plus 0.2 cc (1 mg) progesterone (Prolution, Schering Corporation). Vaginal smears were taken twice daily in order to trace the changes in the vaginal cycle.

During the period of injection each of the five animals was placed with a freshly captured male presumed to be in breeding condition. After the first week daily injections were discontinued, and similar dosages were given at different intervals in order to establish time limits for a complete estrous cycle. The animals were then permitted to recover. All animals were given plentiful supplies of the types of food listed in the following paragraph.

Upon recovery of the animals from the experiment outlined above, three animals were fed morning and evening with nothing but freshly picked green alfalfa. Two other gophers were given only rolled oats. Four additional gophers were given all types of food during the course of the experiment. Vaginal smears were taken twice daily of all animals in order to detect changes in the reproductive cycle. After a month of such treatment all animals were placed on diets containing plentiful supplies of green alfalfa, lab chow, pablum, carrots, rolled oats, and potatoes. Vaginal smears were taken morning and evening for another month.

Observations

During the period of injection with chemical estrogens, all injected females gave evidence of four clear-cut and readily recognizable stages. These are summarized below:

Stage IV — numerous polymorphonuclear leucocytes; occasional epithelial and cornified cells; area around genitalia indistinguishable from surrounding parts. (This is the condition of most animals captured fresh from the field, and in all animals above before injections were begun.)

Stage I—numerous to few polymorphonuclear leucocytes; occasional epithelial cells; many cornified cells; area around genitalia becoming distinguishable from surrounding parts but vulva not turgid.

Stage II—All cornified cells, not in large sheets; vulva turgid; clitoris prominent; entire genital area swollen.

Stage III—few polymorphonuclear leucocytes; no epithelial cells; cornified cells now in sheets; genital area still prominent but less so than in Stage II; vulva not turgid.

The transitions from Stage IV to Stage II were induced by injections of estradiol benzoate in the case of three animals and by estradiol benzoate plus progesterone in the case of two females. As long as

injections continued the animals remained in Stage II. Upon withdrawal of the estrogens Stage II gave way to Stage III and finally to Stage IV. Stages I and II could again be elicited by injections of estrogens, with Stages III and IV following upon withdrawal. It was impossible to establish narrow time limits for the successive stages at the intervals when smears were made. In general, three days after injections were begun the animals were all in Stage II. Two days after withdrawal of the estrogens the females were in Stage IV.

When any of the injected females were placed with a freshly captured male gopher presumed to be in breeding condition during any of the above stages, fighting inevitably followed. Some battles were so severe that the female had to be removed. When fighting was mild, the female was kept with the male overnight or longer. No spermia were detected in the vaginal smears at these times, so mating had evidently not taken place.

Since nothing, therefore, is known of the stage during which actual mating occurs in the gopher, it will not be practical to designate the above stages as diestrum, estrum, etc.

The results recorded from observations on the animals given different diets are summarized in Table 1. As can be seen, diet apparently had no effect in producing changes in the vaginal cycle.

Discussion

Several interesting effects of diet on reproduction have been recorded by different investigators. Moore (9) reviewed some of the effects of nutritional deficiencies, comparable in their scope to the role played by endocrines and other environmental factors in some cases, and resulting in male sterility, or at least in a reduction in fertility. Other investigators could find no support that the materials with which they worked had any effect on reproduction (6, 10). Walker and Wirtschafter (12) established that the rat embryo cannot survive in the presence of a lathyrus factor from a pea-pellet diet beyond the 16th day of gestation. Ershoff (2) showed that the deleterious effects of alpha-estradiol on the immature rat ovary could be counteracted by feeding dried alfalfa.

It has been assumed that the female pocket gopher in irrigated lands in California is polyestrous, breeding throughout the year. In a recent report Gunther (4) stated that pregnant females were captured during every month of the year. Miller (8) reported similarly, and attempted by statistical analysis to establish reproductive cycles and rates in this animal.

The vaginal smear technique is routinely used in testing for estrous cycles in such laboratory animals as the rat and guinea pig. As was indicated above, clear-cut stages are easily recognized in gophers injected with estrogens. Since no criteria exist relative to the determination of an estrous cycle in the female pocket gopher, it was hoped that by artificially inducing such a cycle, readily recognizable stages could be recorded, and such information used in determining the phases of estrum of gophers fresh from the field and under dietary experiments

in the laboratory. If such a cycle, artificially determined, is similar to that in rats, it might be valid to assume that such events occur normally in the estrous cycle of the gopher.

TABLE 1. Observations on animals maintained on different diets.

Animal No.	Weight (gr)	Diet	Vaginal smear (Stage)
1	Varied from 97-124	Green alfalfa	IV
2	" " 94-110	" "	"
3	" " 99-118	" "	"
5	" " 110-120	Rolled oats	"
17	" " 112-117	" "	"
All above	Same variations	All foods	"
20	Varied from 133-162	" "	"
35	" " 133-172	" "	"
39	" " 107-125	" "	"
40	" " 131-155	" "	"

As is indicated from the results of this investigation (Table 1), green alfalfa alone, or in combination with other foods, has no apparent effect on the estrous cycle of the female pocket gopher (if the determination of the estrous cycle by the methods employed be considered valid). This statement must, however, be qualified by a consideration of the conditions under which the experiments were conducted. While every effort was made to simulate the natural environment of the gopher, it should be recalled that the animal was still in an artificially created habitat. While such conditions as temperature and humidity were controlled as much as possible, it by no means follows that the animal in the field would have the same conditions. Furthermore, the amount of digging and the space for roaming severely limited the gopher in terms of exercise in the laboratory. Handling of the animals in order to make vaginal examinations also created another situation which the gopher does not face in the field. It must also be emphasized that although female gophers can be made to produce an estrous cycle through injections of hormones similar to that observed in the natural, and in the artificially induced, cycle in the rat, it by no means follows that these gophers undergo the same cycles in their natural environment.

Since these animals spend 99 percent of their time underground, it is possible that instead of eating green alfalfa (the parts of the plant above ground) they exist on nothing but the roots. The factor controlling the estrous cycle might lie resident within the roots. This possibility was not probed in this investigation. It has been observed, however, that gophers frequently pack their burrows with green alfalfa. On the other hand, fields of alfalfa have been noted with isolated stands of blighted, shrivelled, and yellowed alfalfa stalks. When such areas were examined carefully, it was noted that gopher diggings were

numerous throughout the stand, and when the alfalfa plant was pulled from the ground it was seen that the roots had been entirely gnawed away.

A possibility exists that the female gophers under consideration were already pregnant, in which case a Stage IV would normally appear in the vaginal smear. Upon autopsy of the animals, no evidence of pregnancy was noted. Fresh placental scars or corpora lutea were absent. There was no indication of recently resorbed embryos. Furthermore, if resorption of the embryos had occurred, there should have been ample time for recovery of the uterus and re-establishment of a normal sexual cycle.

Finally, it may be postulated that psychological factors intervened, thus effectively preventing the appearance of a normal estrous cycle and consequent laboratory matings of these animals. Even when given a normal environment, or as nearly normal as laboratory conditions permit, gophers inevitably fight when placed together in a cage in the laboratory. This was true when females were injected either with estradiol benzoate alone or with estradiol benzoate plus progesterone. Regardless of sex, the stronger (larger) usually attempted to kill the weaker (smaller).

Summary

1. An artificially induced estrous cycle has been described for the female pocket gopher.

2. Females injected with estradiol benzoate, or with estradiol benzoate plus progesterone, did not mate with freshly captured males in the laboratory.

3. Animals maintained on diets of fresh alfalfa alone, or with lab chow, pablum, rolled oats, carrots, and potatoes added, show no indications of undergoing a normal estrous cycle under the experimental conditions listed.

4. It is postulated that factors other than diet alone are responsible for the abandonment of the estrous cycle of female pocket gophers and their consequent failure to mate in the laboratory. Such factors may be psychological.

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