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Evaluation of the Gross Anatomy Seasonal Changes Function and Histology of the Perineal Gland in the Hispid Cotton Rat, *Sigmodon hispidus*

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by

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ABSTRACT

Evaluation of the Gross Anatomy, Seasonal Changes, Function and Histology of the Perineal Gland in the Hispid Cotton Rat, *Sigmodon hispidus*

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The perineal gland in the hispid cotton rat, *Sigmodon hispidus*, is a seasonally cyclic, subcutaneously located organ which is associated with the reproductive system. On gross dissection, the gland possesses a strong attachment to the penis with only loose fascial connections to the rectum and surrounding muscle and skin. Histologic exam confirms this connection to the reproductive tract and shows the organ to be a compound tubuloacinar gland with a projection leading into the penis. The cyclic hypertrophy and regression of the gland closely parallels that of the testes and seminal vesicles indicating that this cyclicity may be under androgen control. The weight of the gland also shows extremely high correlation to the weights of these other reproductive organs (Pearson's Correlation Coefficient to testes weight = 0.933 and to seminal vesicle weight = 0.930; $P < 0.0001$). Field testing to determine the function of the glandular product and its effect on wild populations initially indicated an attractant effect on males within the area when trapping was done near the end of the breeding season. An additional test was performed in an attempt to determine the chemical nature of the active component of the glandular secretion. Chemical extraction of the glandular product was done and field testing was established to determine the effects of the different fractions on wild

populations. The results of this test proved inconclusive indicating a need for additional studies into the function of the gland.

ACKNOWLEDGEMENTS

I would like to recognize the encouragement and assistance that I have received from many friends, advisors, employers and peers, without which, I might never have committed myself to completion of this course of graduate study. I am especially grateful to Bruce Averitt for believing in my abilities and encouraging continuation of my studies after so many years. The enthusiasm which Dr. Gerald Pepe exhibited can be attributed with pushing me on into a graduate program before even more time had elapsed, and for that I am grateful.

There were so many dedicated scientists who assisted me in various aspects of my research that it will be difficult to list them all. However, I do want to thank Dr. Patricia B. Williams, Jim Merritt, Patty Loose, Chris Pague, April Yoder and Mike Adams. Dr. Williams always supported my research in spirit even though the time I spent in my own work cut into the time which I could devote to work in her lab. She also graciously allowed me the use of her lab space and equipment which made my life as a graduate student easier. The ideas, camaraderie and assistance in field studies that were provided by Jim Merritt were invaluable and have placed him in my heart as a lasting friend. The instruction and assistance I received from Chris Pague and Patty Loose while I was fighting with histology sectioning was greatly appreciated and will not be forgotten. The assistance that April Yoder provided in my initial field trapping study was a godsend, especially on the morning of an unexpected snow fall and when the U2 tickets went on sale. Of course no graduate study is complete without slides and

figures and the hard work that Mike Adams put into this final product is a testament to his abilities.

Lastly, I would like to thank the members of my committee. The experience that I have gained from Dr. Rose in the field and at the necropsy table will serve me well. His editorial eye and insistence on a quality product have also helped to make this thesis more informative and readable. The assistance that Dr. Carson provided with the perfusion and histology studies certainly expanded my knowledge in that field and his comments assisted in clarifying some of the results for me. The benefits that I received from Dr. Stevens went well beyond the histologic and photographic expertise that he provided. His encouragement and understanding of my difficulties with balancing a career, graduate school and life helped me get through.

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INTRODUCTION

Sigmodon hispidus, the hispid cotton rat, is a native, nocturnal rodent which inhabits old fields and early successional habitats throughout the southern United States. Its northern range limits have advanced into southern Virginia (Patton, 1941) and Kansas during its rapid range expansion of the past 50 years. Although primarily an herbivore consuming grasses, herbaceous plants and seeds, the cotton rat will also consume insects during the summer months (Fleharty and Olson, 1969). This opportunistic feeding pattern allows cotton rats to inhabit marginal habitats including successional areas shortly after disturbance. This ability and their vagile dispersing nature have allowed for their remarkable recent range expansion, apparently in response to changing land-use patterns.

In addition to their flexible feeding habits, their reproductive strategy also facilitates a dispersing lifestyle. Cotton rats are seasonal breeders with a breeding season in southern Virginia from late February to early October (Rose and Mitchell, 1990). The onset of male reproductive capacity precedes the female reproductive readiness by two to three weeks in the late winter and continues beyond the breeding season of females in the fall. In Virginia, the female gives birth to 5-6 precocial pups per litter (Rose and Mitchell, 1990). The pups are weaned in 20 days and are capable of reproduction by 50 days. Because of this large reproductive capacity the

population numbers are capable of dramatic swings. This high reproductive potential has prompted further studies of reproductive processes.

While studying the reproduction of *S. hispidus*, Dr. Robert Rose noted the presence of a large subcutaneous organ near the base of the tail in adult male cotton rats. The size of the organ increased with the initiation of the breeding season and regressed to an undiscernible size during the non-breeding winter months. Although prominent in adult breeding males, the organ was not noticeable in females. In order to evaluate the nature of this organ, I undertook studies of the gross anatomy, histology and behavioral effects of secretions of the gland. Initially the perianal location of the gland and the proximity to the rectum suggested that the gland would function as an anal gland. However, on closer inspection the gland shows associations throughout the perineal region; therefore, I will hereafter refer to this organ as the perineal gland.

Literature Review

The importance of mammalian olfaction for hunting, food gathering and predator avoidance has long been recognized. However, it has only been within the last 100 years that the importance of olfaction in mammalian communication has begun to be uncovered. Olfactory signals are used by mammals to communicate a variety of information about and between mammals living within the same habitat. These chemical signals can give information on the territorial boundaries of an individual or group, the social position of an animal within a group and the reproductive status of an individual. In addition, chemical signals can be used to

transmit warning signals between conspecifics (Stoddart, 1977). These various signals can alter the behavior and the physiology of mammals, thereby improving their chances for survival.

In larger mammals, where acoustic and visual messages are also used, olfactory cues can be given in combination with visual signs for transmission of danger and warning signals. Several species, including the pronghorn antelope (*Antilocapra americana*) the rock cavy (*Dendrohyrax dorsalis*) and the Patagonian hare (*Dolichotis patagona*), all possess patches or bands of specialized white erectile hairs which are erected when the animals are alarmed (Stoddart, 1977). In addition to the visual signal of these flashes, the erection of the hairs also broadcasts a chemical secretion from specialized glandular tissue associated with the areas adjacent to or under the white patches. In this way both olfactory and visual signals inform conspecifics of possible danger or changes within their environment.

Olfactory chemicals that are broadcast through pilo-erection may serve other non-warning functions. This is believed to be the case with the crested rat, *Lophiomys imhausi* (Stoddart, 1979). When the crested rat is alarmed, the crest of hairs from the nape of the neck to the tail rises. Erection of the hairs emits the scent of the glandular secretion with which the sponge-like hairs are saturated. This strong odor is thought to repel predators in much the same way that the mist sprayed by skunks protects them. Pilo-erection may also facilitate other non-warning odor messages since the use of pilo-erection is not restricted to alarm situations. At this time, however, not enough research has been done to understand the nature and information content of these more subtle signals.

In small mammals, olfactory perception probably plays an even more important role in communication than in the larger mammals. Because large mammals can use visual and auditory

communication, olfaction is a supplemental source of information. However, in small mammals the denseness of the vegetation within their habitat often reduces the effectiveness of either hearing or vision (Stoddart, 1974). Because of this, olfaction plays a primary role in many aspects of the lives of small mammals.

The effect of scents in the natural environment is difficult to document. However, many population studies in small mammals have noted an influence of olfaction on the ability to accurately determine population dynamics. Early studies showing that the use of soiled traps greatly enhanced the trappability of some species were corroborated by later studies. Boonstra and Krebs (1976) found that *Microtus tounsendii* will enter dirty traps more often than clean ones. This effect is more pronounced in voles captured for the first time, in young voles, and during the breeding season. In another species of vole, *Microtus agrestis* L., Stoddart (1982) found that not only did voles prefer a trap with odor present, but they could distinguish between their own and another vole's odor and preferred traps soiled with their own odor. The second most common trap preference was that of adult males for traps containing the residue of female odor (Stoddart, 1982). This preference for the scent of the opposite sex also occurs in *Peromyscus leucopus*, where both sexes preferentially seek out scents from the opposite sex (Mazdzer et al., 1976). Contradictory studies in field trapping of the cotton rat, *Sigmodon hispidus*, showed no trap preference in reproductive males and a significant preference for clean traps in nonreproductive males and females. Reproductive females, however, showed a definite preference for soiled traps (Summerlin and Wolfe, 1973). These differences in trap preference between species and even between sexes and reproductive status within a species indicate the complex role of olfaction in small mammals.

In addition to the changes which scents can evoke in trap response, physiologic changes can also result from olfactory stimuli. As early as 1959, Whitten reported on the effect of scents on the estrous cycles of house mice. When female *Mus musculus* were housed in groups, they became anestrus. This effect could be seen in intact mice, blinded mice and mice that were partitioned off by either solid or perforated partitions, showing that visual or tactile signals did not affect the impairment of regular estrous cycles.

Not only do odors from females affect one another, but male odors can have a dramatic effect on female reproductive physiology. The Bruce effect (Bruce, 1960) results in failed pregnancy when recently mated female mice are housed with or near strange males. Since physical contact between the male and female is not necessary, male pheromones almost certainly cause the endocrine changes which induce the failure of pregnancy and return to estrus. In addition to the Bruce effect, Vandenbergh (1976) has shown that male pheromones can accelerate sexual maturation in female mice and rats. Early onset of puberty in females is most pronounced when young females are housed singly with adult males. However, slightly earlier maturation also occurs in females living in male-inclusive groups as compared to isolated females. Females housed together have the slowest rates of sexual maturation, showing the inhibitory effect of the presence of females on the sexual maturation of other female mice (Vandenbergh, 1973).

These physiological studies show that the social setting in some rodents plays an important role in pheromonally induced physiologic changes. Studies on the group structure and the position of the individual in the social hierarchy show that social position can alter the effect of pheromones. For example, in house mice the urine from dominant males will induce the early

onset of puberty in females, but females show no acceleration of puberty when exposed to urine from subordinate males (Lombardi and Vandenbergh, 1977). These studies, however, were done under laboratory conditions and their relevance to natural populations has yet to be determined.

One study done under semi-natural conditions in island populations of feral mice was reported by Massey and Vandenbergh (1980). The study showed that population density affects the pheromone production in female mice. Urinary pheromones from female mice in a high-density feral population were able to delay puberty in laboratory mice. However, urine from females from an adjacent low-density population did not produce this effect.

These field and laboratory studies show that mammals produce chemical odorants for release into their environment which elicit specific physiologic and behavioral responses. These odors may serve to help control the mammalian population numbers, spacing, social behavior and use of habitat. The importance of these chemicals to survivability, reproduction and population structures may have allowed for the natural selection of animals with the most successful odor adaptations. Natural selection for more effective odors has promoted the enhancement and inheritance of these traits. This resulted in the development of further specialization of scent-producing glands and organs and the development of strategies to relay this olfactory information to other animals.

As a result of this selection many mammals possess specialized systems for placing odorants in their environments. Urinary, fecal and sebaceous odorants produced in specialized tissues and glands serve as repellants, attractants, modifiers and identifiers. In insectivores and rodents, at least six zones contain specialized sebaceous tissue (Stoddart, 1974). Modified

sebaceous glands which open onto the surface of the skin are found in the flank, sternum, head, tail, rump and mid-dorsal region. These are usually holocrine glands but may have associated apocrine glands. In the rabbit (*Oryctolagus cuniculus*) scents from anal, chin and inguinal glands are all used for communication. The relative size and volume of production of these scent glands relate to the age, sex, social status and reproductive condition of the individual and are hormonally controlled. Chin and anal secretions are used for territorial marking but the scent from the inguinal gland is used for individual identification (Goodrich and Mykytowycz, 1972).

In addition to these specialized structures of the skin, many species have developed glands associated with the urinary and alimentary tracts. The beaver (*Castor canadensis*) has two pairs of large glands near the tail. The anal glands exude contents directly onto external papillae. By contrast, the castor glands join the urethra and open into the reproductive tract (Svendsen, 1978). The products of these glands are deposited at scent mounds, thus marking territory boundaries (Aleksiuk, 1968). Other species have also developed specialized glands associated with fecal scent-marking such as the proctodael glands in *Microtus agrestis* (Khan and Stoddart, 1988). As in the chin gland of the rabbit, these glands are sexually dimorphic and respond to hormonal control (Khan, 1984). The scent produced by field voles is probably also used in territorial identification since it has a definite primer effect on the gland enlargement of other males (Khan and Stoddart, 1986). At this time, however, field studies on natural populations have not been performed.

These specialized secretions are produced and placed in their environs to provide information to conspecifics. With beaver and rabbit, specialized group marking within the home territory occurs. All members of a group contribute to the scent-marking; however, dominant

animals, especially males, mark most frequently. These marking techniques are seldom exhibited outside the home range and the scent-mark of the home territory has a repellant effect on animals from outside the group (Mykytowycz, 1965 and Aleksiuk, 1968).

Social influences, although not observed in the field, can be implied in *Microtus agrestis*. Since the social environment of this vole has been demonstrated to alter the size and function of the proctodeal gland (Khan and Stoddart, 1988), it may be assumed that the secretory product of this gland, when emanating on fecal pellets within the territory, emits chemical messages to other conspecifics. Whether these messages are used as repellants, attractants, inhibitors or social qualifiers is still unclear because of the difficulties of studying the behavior of small animals in the wild.

As previously mentioned, these specialized scent-producing tissues are often sexually dimorphic, seasonally regressive and the size and production of glandular products usually are under hormonal control. Mykytowycz (1965) concluded from castration studies in the rabbit that not only the size of the scent-producing chin glands are reduced by castration of males, but also the volume of secretory product from this apocrine gland is lowered. Conversely, ovariectomized female rabbits show an increased volume of apocrine secretion. His studies also showed that the chemical composition of the odorous secretions also changes with changes in the hormonal status of the animal (Goodrich and Mykytowycz, 1972). Males, females and pregnant females all show unique chemical compositions of secretions from the anal, chin and inguinal glands. Clear differences exist in the hydrocarbon composition between various glands in males and females, as well as sex differences within the same type of gland. Their study showed that concentrations and proportions for proteins and carbohydrates bound to proteins found in pure secretions of the

chin and anal glands varied between sexes. Protein-bound carbohydrates, although found in all of the secretions, are a major constituent only in secretions of chin glands of male rabbits. Because these glands seasonally regress, their secretions are used heavily by breeding males for territorial marking and their size can be reduced by castration, it can be concluded that the glands are controlled by reproductive hormones.

Unlike Mykytowycz's castration studies, other studies evaluating the role of androgen control of scent glands have used introduction of exogenous steroids to determine the androgenic effects. In the supracaudal gland of the male guinea pig (Martan, 1962), the midventral sebaceous gland in the Mongolian gerbil (Thiessen et. al., 1968) and the proctodael glands of *Microtus agrestis* (Khan, 1984), castration will reduce the size of the gland, or in juvenile males prevent the initial growth of the glandular acini. Furthermore, replacement injections of testosterone propionate in castrated males will cause enlargement of the gland and production of normal amounts of sebum. These studies clearly defined the role of testosterone in the control of these scent-producing organs.

Objectives of the Present Study

The existence of specialized tissues and controlling mechanisms for odor production and their wide array of functions have prompted further investigation of their nature and use in other species. Initial observations of a seasonally regressive, extremely large perineal tissue in male cotton rats led to investigations into the glandular nature of the tissue, its seasonality, method of product delivery and effect on conspecifics in a natural setting.

The six specific objectives established for the study were to: 1) describe the gross anatomy of the perineal gland in sexually active males, 2) describe the annual cycle of growth and regression of the perineal gland using samples of mature males collected on a monthly basis throughout the year, 3) examine the correlation of the perineal gland growth/regression with the annual cycle of growth and regression of the testes and seminal vesicles, and with other standard measurements, 4) examine the glandular tissues using standard histological methods, 5) determine the method of delivery into the environment using histological analysis, 6) learn the information content of the perineal gland in the hispid cotton rat by placing crushed active glands and their extracted compounds into the natural environment and measuring the responses of conspecifics in the wild.

MATERIALS AND METHODS

Laboratory Studies

Specimens of hispid cotton rats to be used in lab studies were obtained using Fitch live traps placed approximately 5 m apart on a transect line placed in old fields in southeastern Virginia. For studies of the gross anatomy and seasonality of the perineal gland, only adult males > 50 grams were euthanized with chloroform while in the field. The specimens were then removed to the lab and frozen until necropsies could later be performed. Specimens were obtained at the Portsmouth refinery site in Portsmouth, VA, Ragged Island in Isle of Wight County, VA, on Virginia Department of Highway and Transportation (VDOT) property located at the Highway 58 interchanges in Holland and Chesapeake, VA and the Joliffe Road (VDOT) site in Chesapeake, VA. A metric rule was used for measurements and a Mettler PL200 was used to determine the weights of animals and their organs.

In order to prepare tissues for histologic examination wild-caught animals were initially anesthetized with ether in the lab. This was followed up with an intraperitoneal injection of 0.66 to 1.0 ml of tribromo-ethanol (1 gm/40 ml) to maintain anesthesia during the perfusion. The thoracic cavity was opened, a cannula was placed in the left ventricle for introduction of the perfusate and the inferior vena cava was cut to allow the exit of fluids. The tissues were initially

flushed with heparinized buffered saline, then perfused with a solution of 4 percent paraformaldehyde in 0.1M phosphate buffer (Glauert, 1975). Next, the perineal region was dissected away from the remainder of the lower torso and stored in 10 percent formaldehyde fixative for 24 hours. At that point the pelvic girdle and spine were carefully removed and the remaining tissue was placed in 10 percent formalin until prepared for embedding approximately 20 hours later.

Due to the large size and oily nature of the tissue when fully enlarged, a 75 g male with scrotal testes was sacrificed at the onset of the breeding season. At this time the gland is only partially enlarged and was therefore small enough to allow dehydration and fixation of the tissue in situ rather than requiring removal of the perineal gland from the surrounding structures. The initial paraffin block was formed from the pelvic girdle and associated lumbar and caudal vertebrae. Later, this block was divided into three smaller pieces after removal of the ossified components and before dehydration. The caudal piece included the majority of the gland, the central piece included a large portion of the penile shaft and the third and most craniad piece, which extended into the pelvic cavity, included the external penis, seminal vesicles and preputial glands. These samples were placed for 30 min in successive baths of 25, 50, 70 and 80% ethanol for dehydration before being placed in an Autotechnicon 2A automated tissue processor for overnight paraffin embedding. After processing, the paraffin blocks were thin-sectioned with American Optical AO 820 microtome. The 10u sections were stained with either a modified Periodic Acid Schiffs (PAS) or a Mayer's hematoxylin and eosin (H & E) stain (Luna, 1968).

Field Studies

In these studies, perineal gland tissue was obtained at necropsy by gross dissection from several fully mature males was stored at -20°C until it was processed and used for live-trapping studies to determine its information content. The tissue was thawed, weighed and minced before homogenization with a Brinkman homogenizer. For the first study a total of 12.3 gm of perineal gland tissue from the pooled sample of several adult breeding males was homogenized in 45 ml of a 70% ethyl alcohol, 30% phosphate-buffered saline (PBS) solution which contained 200 mg/L of L-ascorbic acid. (The ethyl alcohol was used as an organic solvent to solubilize any lipids present, the PBS was used to stabilize the pH and the L-ascorbic acid was used to inhibit oxidation.) This solution was also used as the control for the study. Both homogenate and control solutions were then stored at -20°C until used in the field studies.

This perineal gland slurry was then used as a scent bait in experimental traps, with the ethyl alcohol/PBS solution being used for the control traps. The homogenates and controls were taken into the field and placed in the traps by saturating 4.2 cm x 4.9 cm triangles of Watman #11541 filter paper in either the homogenate or the control solutions, and then placing the saturated paper in the appropriate trap.

The experimental plot was established on Nov. 18, 1987' as a 6 x 5 grid with each trap being placed 7.6 m apart. The placement of the 15 control and 15 homogenate baited traps was determined by randomly drawing lots. Before the experiment, the plot was trapped for 19 days during September and October to determine a population estimate and the sex ratios and reproductive status of the population. Once baited with filter papers saturated with either the perineal gland homogenate or the solvent control, the traps were checked daily. The saturated filter papers were replaced with new papers every third day. The experiment was run for ten

days (through Nov. 29th), during which animals captured were evaluated for their sexual status, weight, foot and ear length. This experiment was conducted at the start of the non-breeding season.

An additional study, conducted near the end of the breeding season in September 1988, examined the effect of chemically separated fractions of the perineal gland homogenate on wild populations. For this study, perineal gland and muscle tissue (from the flanks of male cotton rats) were homogenized separately in PBS (1g/4ml). These homogenates were extracted three times with a 9:1 chloroform:methanol solution. The top (methanol/water) fractions were kept at -20°C until use while the bottom (chloroform) fractions had the chloroform evaporated off under a gentle nitrogen stream and were then reconstituted in a volume of methanol equal to the volume of the top fraction.

For the field study to evaluate the information content of these fractions a 90 trap (6 x 15) grid was established at the Hwy 58-460 interchange in Chesapeake, Va. Fifteen traps were assigned to each of the six treatments in a stratified random fashion. Each trap was baited with a 0.1g sample of either muscle or perineal gland homogenate, or a chloroform or methanol extract (extracted from 0.1 g of homogenized tissue). These samples were placed in the field on triangles cut from #10 Whatman filter paper as before. The traps were checked daily and freshly saturated filter papers replaced the old papers after the fifth trap night. The experiment was continued for ten trap nights. Comparisons of the size, mass and sexual status of the captured animals were again made.

Methods of Analysis

The SAS statistical package was used for determination of the means, ranges, standard errors, analysis of variance with stepwise regression and Pearson's correlation coefficients in the gross anatomy and seasonality studies (SAS Institute, Inc., 1985). Pharm-test (Tallarida and Murray, 1981) was used for determining Chi square, means and Student's t-tests for analysis of the effect of the perineal gland on trappability.

RESULTS

Location and Description of the Perineal Gland

The perineal gland complex is located subcutaneously in the pelvic region above the testes and just anterior to the anus; it literally rings the rectum. Figure 1 shows this area from a ventro-medial aspect in a mature male cotton rat after scrotal incision and retraction. The figure shows the reproductively functional, enlarged testes which were descended in the darkly pigmented scrotum. The testes were excised in order to see the position of the gland with respect to the rectum, penis and gracilis muscle of the hind legs (Fig. 2). The most anterior aspects of the gland originate at the base of the penis and extend dorsally beyond the fourth caudal vertebra.

The size, weight and shape of the perineal gland can vary dramatically depending on the breeding status, which is determined by the age of the animal as well as the season. When the male is in the non-breeding condition the gland complex is greatly reduced and frequently difficult to discern. As the breeding season approaches glandular hypertrophy occurs. Six definite regions can then be observed, with the overall shape being similar to that of a red blood cell with its symmetrical biconcave shape (Figs. 2 & 3). Two lung-shaped lateral regions are distinctly separated from the body of the gland. These elliptical portions have a lobular

Figure 1 Ventro-medial (pelvic) view of sexually mature reproductive male cotton rat (*S. hispidus*) at 15.5 x magnification. Convolutions of the epididymis indicate the presence of mature sperm, and hence fertility.
1) superficial caudal fascia and rectum 2) epididymis 3) left and right testes 4) penis/penile sheath

FIG. 1

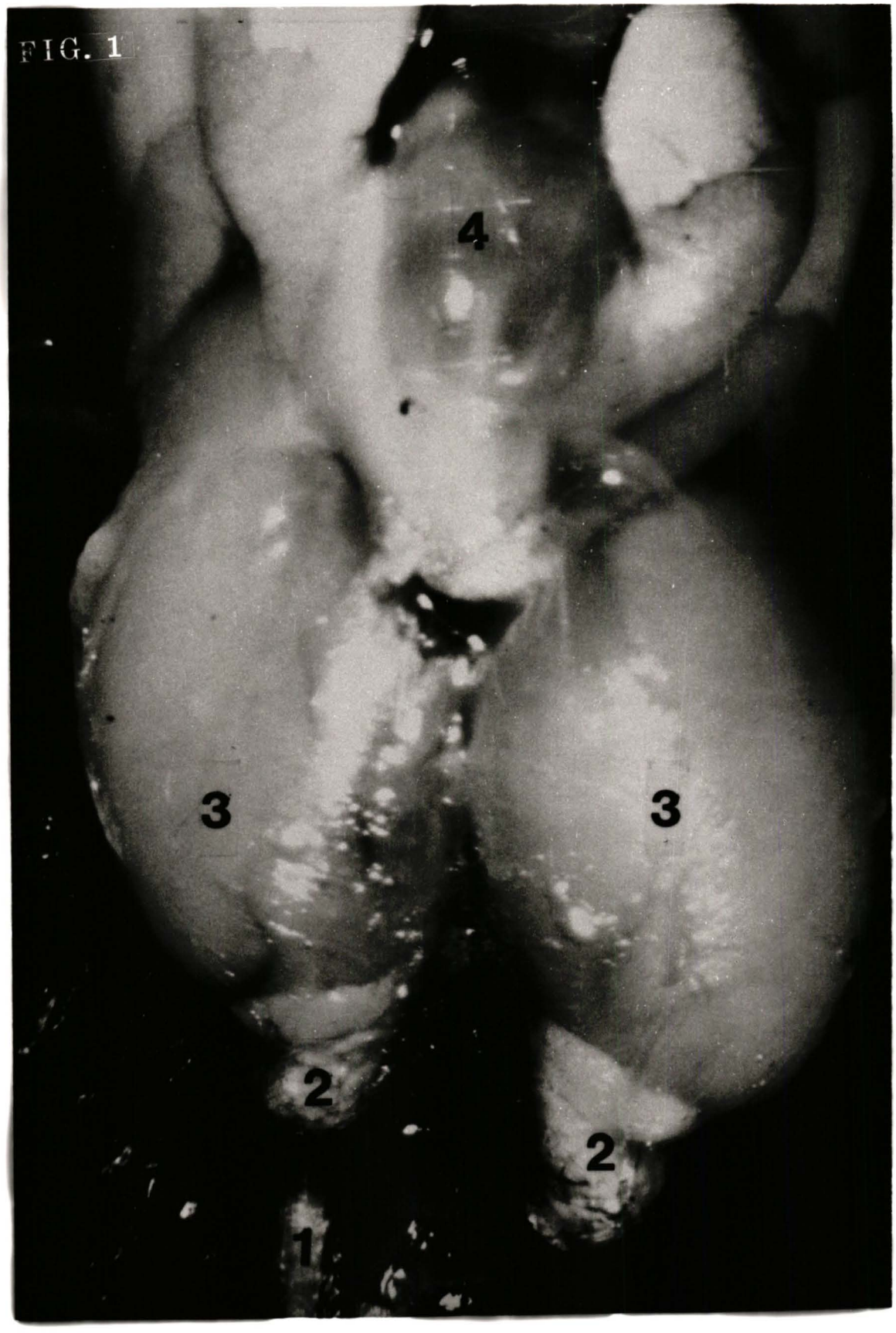


Figure 2 Deep ventro-medial (pelvic) view of sexually mature male cotton rat with testes excised to reveal the perineal gland which surrounds the rectum at 17.7 x magnification. Note the lobular shape and the apposition of the anterio-cranial part of the perineal gland to the penis.
1) superficial caudal fascia and rectum 2) perineal gland 3) penis 4) gracilis (skeletal) muscle

FIG. 2

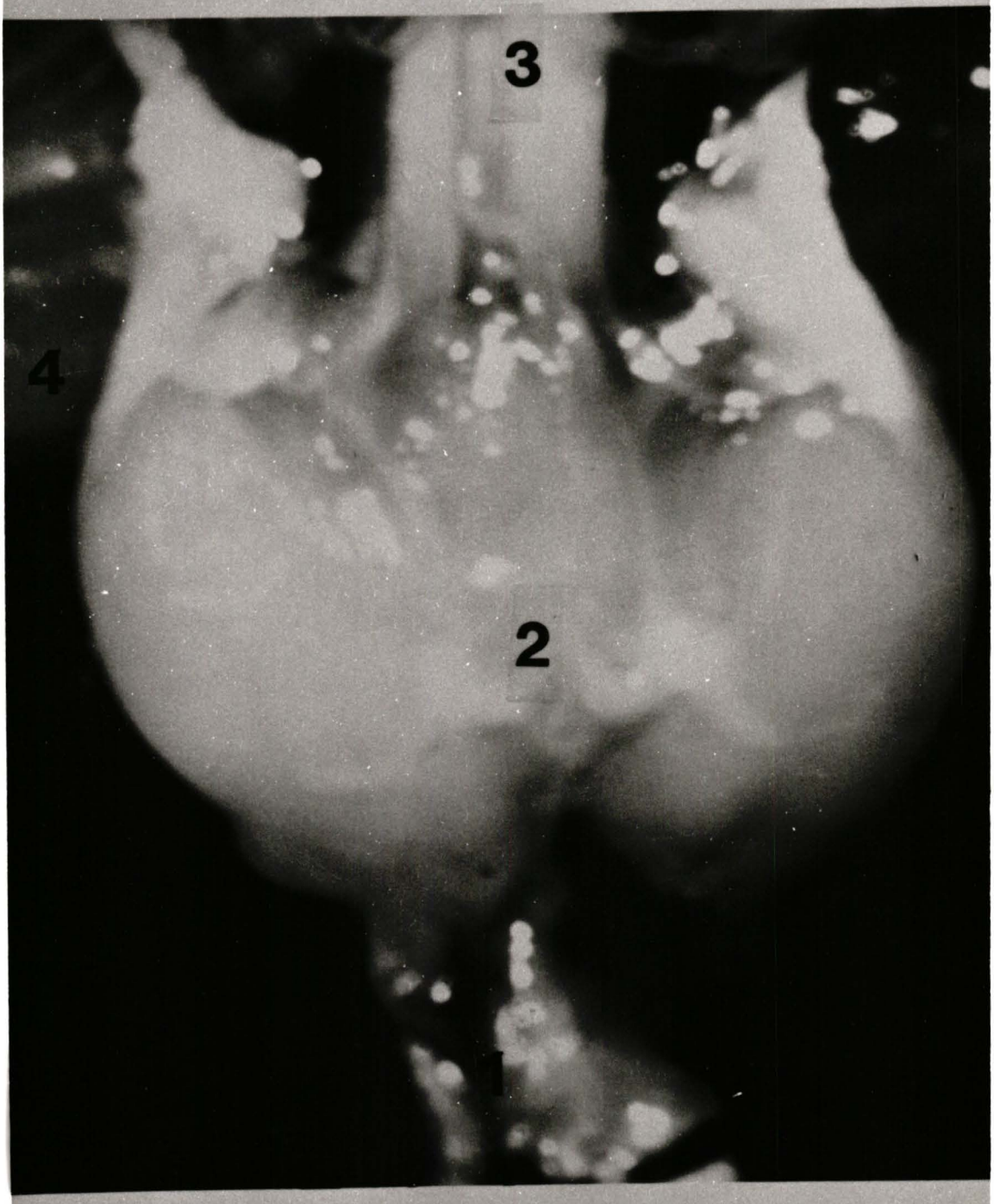
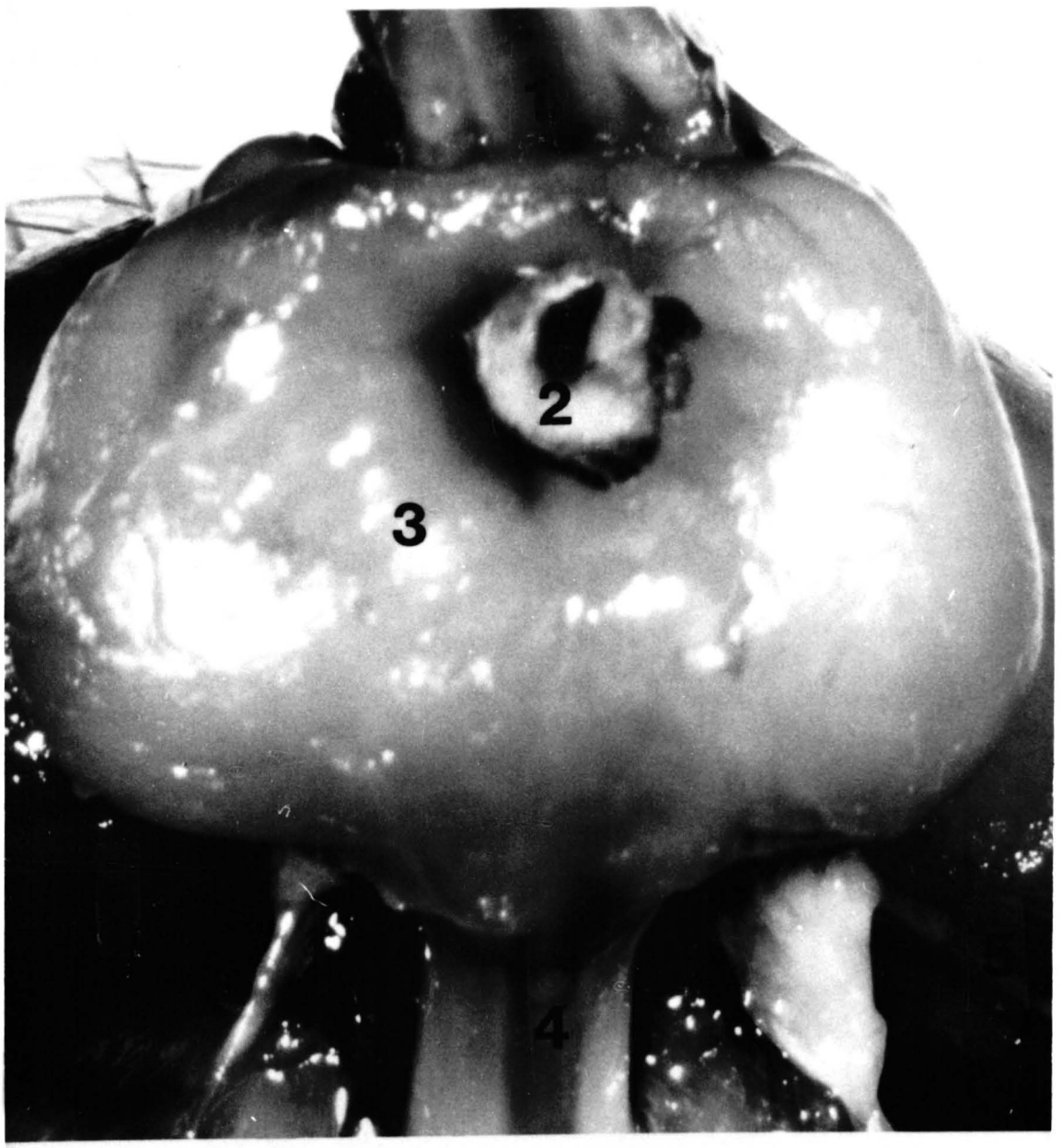


Figure 3 Posterior dorsal view of sexually mature cotton rat showing the perineal gland encircling the severed rectum at 13.1 x magnification. Testes were excised to expose the perineal gland. 1) tail 2) rectum 3) perineal gland 4) penile shaft 5) gracilis (skeletal) muscle


2
3
4

appearance and dorsally they flank either side of the rectum (Fig. 4). The lobes are held in place adjacent to the glandular mass by a single fibrous tubule (observed but not characterized histologically) and elsewhere by loosely attached fascia. Due to the similarity of placement and description of the Cowper's gland of the porcupine (Mirand and Shadle, 1953), it is possible that these paired lobes are not part of the perineal gland, but are actually Cowper's glands.

The larger glandular mass is less distinctively segmented, but appears to be divided into four regions. The four major segments of the gland encircle and are attached by loose fascia to the rectum (Fig. 3). The segments located anterior and ventral to the rectum are the largest and have strong, definite attachments to the base of the penis (Fig. 5). The two remaining smaller segments are dorsal to the rectum and caudal to the penis attachment points of the larger segments. The lung-shaped lobes are located beside them (Fig. 4).

Seasonality of the Perineal Gland

Because the size and weight of the perineal gland complex vary dramatically with the seasons, animals were collected throughout the year to document these changes. In all, 129 male cotton rats were evaluated and the raw data is shown in Appendix A. At necropsy overall condition of the animal was assessed and the sexual status determined. Standard body measurements were taken including total body length, tail length, body length (total body length minus tail length), hindfoot length and ear length. In addition, the total body weights, testes weights, seminal vesicles weights and perineal gland weights were determined.

Figure 4 Side view of sexually mature male cotton rat showing the lobular and protruding perineal gland at 16.6 x magnification. Note the extension of the enlarged gland well behind the thigh muscles. 1) superficial caudal fascia 2) rectum 3a, b, c) lobes of perineal gland 4) biceps femoris (skeletal) muscle 5) semitendinosus (skeletal) muscle

FIG. 4

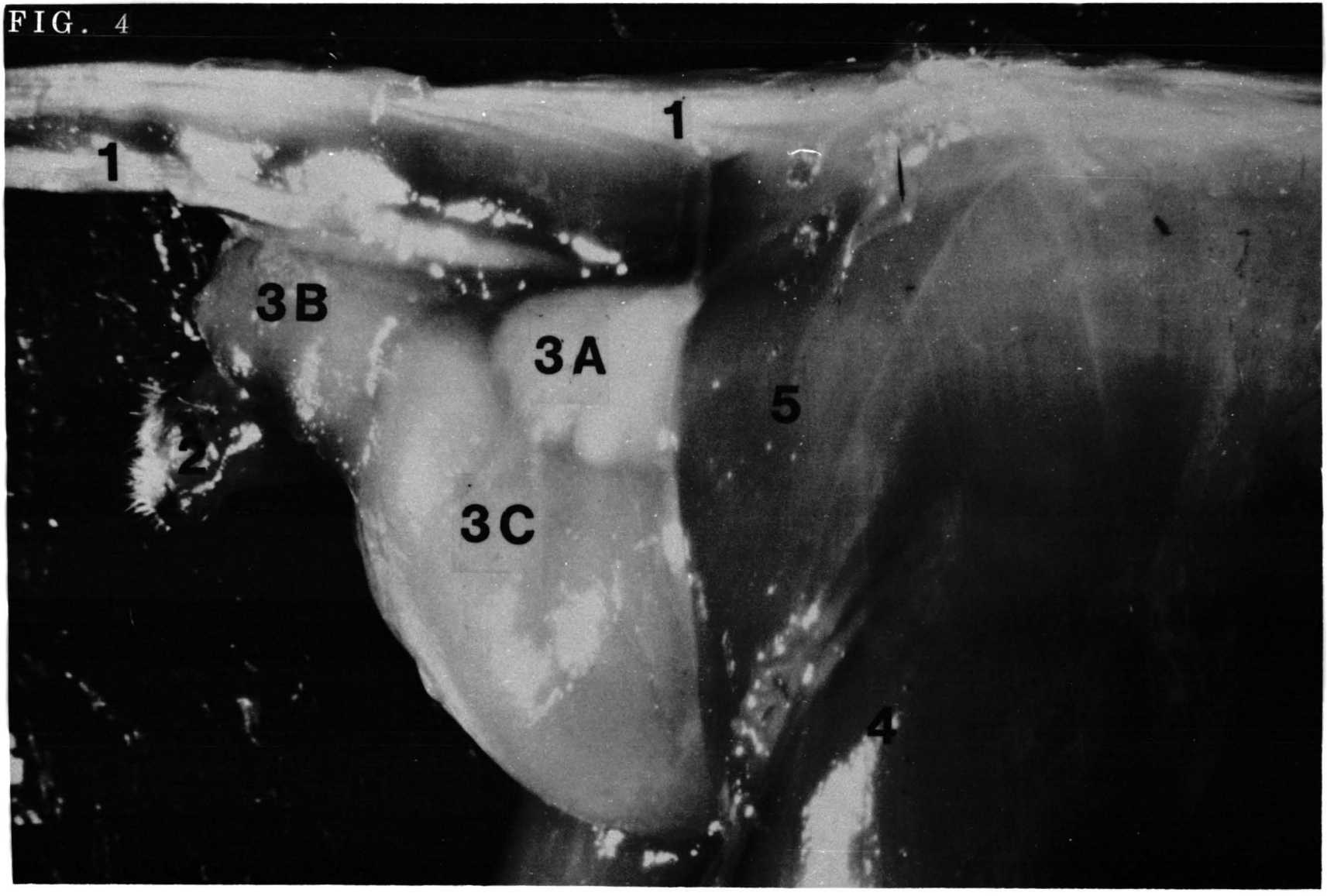


Figure 5 Ventro-medial pelvic view of the sexually mature male cotton rat after excision of testes at 15.6 x magnification. The perineal gland has been retracted from its position over the penile shaft to show the strong ligamentous attachments.
1) perineal gland 2) penile shaft 3) gracilis (skeletal) muscle 4) glans penis 5) remnants of epidermal covering

FIG. 5

Comparing monthly samples, the mean weight of the perineal gland decreased dramatically in October. By November the gland was undetectable by gross dissection and remained so until February (Fig. 6 A) when glandular hypertrophy was initiated. The size continued to increase through May and June. Another sharp but temporary drop in mean weight occurred during July. This may be due in part to young males entering the population, causing a change in population structure resulting in lower mean weights as well as lower weights of the perineal gland and reproductive organs. Plots of the perineal gland weights versus the testes and seminal vesicles weights for the annual cycle and individual months are included in Appendix B. These scattergrams were used to visualize the populational structure within the months, as well as the changes occurring over time.

In order to illustrate the variability and relative importance of the perineal gland, the ranges, means and standard errors for the means for reproductive organs and other body measurements were determined (Table 1). Means and ranges were determined for the entire annual sample, for non-breeding season samples (November through January) and for breeding season samples (February through October). The results showed the uniformity in size and mass of the animals throughout the year. They also indicated the dramatic difference in the weights of the perineal glands, testes and seminal vesicles between the breeding and non-breeding samples. When a general linear model analysis of variance was used to compare the breeding and non-breeding season samples all three organs showed a significant difference in weights between seasons at the $P = 0.05$ level with one degree of freedom (perineal gland F value = 67.65; testes F value = 101.34; seminal vesicles F value = 45.48). The striking seasonal change in the weight and form of the perineal gland alludes to its potential importance. Even

Figure 6 Comparison of mean monthly weights of the perineal gland (A), testes (B) and seminal vesicles (C). N = 129.

FIGURE 6

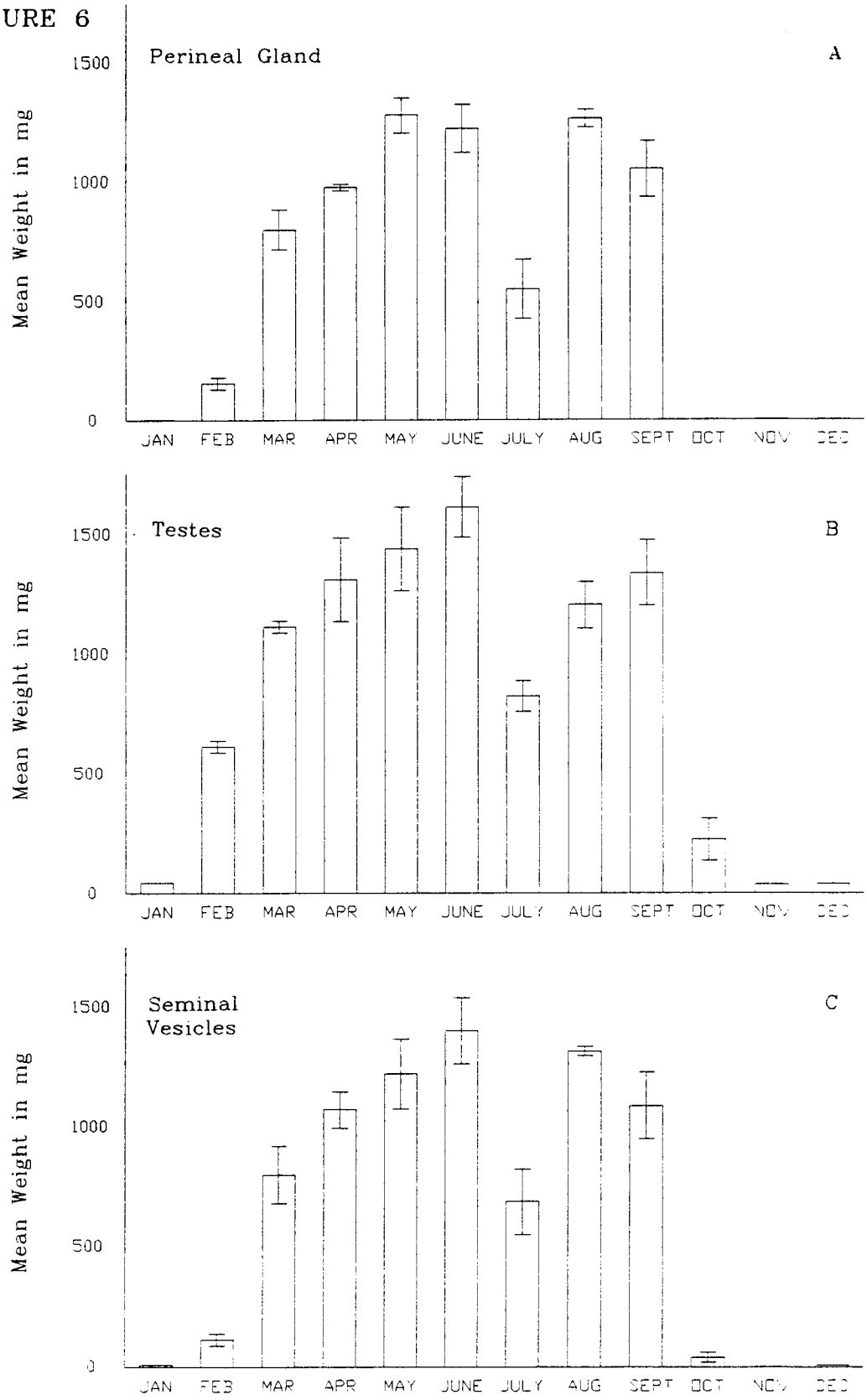

Perineal Gland
A
Testes
B
Seminal Vesicles
C
Mean Weight in mg
1500
1000
500
0
JAN
FEB
MAR
APR
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
DEC

Table 1 Means and variability for weights of perineal gland, testes, and seminal vesicle and for body measurements for adult male cotton rats (>50 grams) collected each month throughout the year. The non-breeding season includes the months of November to January (when the perineal gland is too small to detect) and the breeding season extends from February to October. Body length is the total length minus the tail length.

	ANNUAL N=129		NON-BREEDING SEASON N=39		BREEDING SEASON N=90	
VARIABLES	MEAN	RANGE	MEAN	RANGE	MEAN	RANGE
Perineal gland (mg)	568 ± 55.7	1-2342	1.0 ± 0.0	0	813 ± 65.2	1-2342
Testes (mg)	776 ± 63.6	20-2311	44.5 ± 2.7	20-104	1093 ± 68.4	38-2311
Seminal vesicles (mg)	579 ± 64.6	1-3080	6.8 ± 0.8	1-15	827 ± 80.0	1-3080
Body length (mg)	145 ± 1.3	114-185	137.3 ± 1.8	114-160	148 ± 1.7	116-185
Body weight (g)	97 ± 2.3	51-168	88.3 ± 3.2	52-121	101 ± 2.8	51-168
Hindfoot length (mm)	30.2 ± 0.1	26-35	29.9 ± 0.3	26-35	30 ± 0.1	27-33
Ear length (mm)	17.8 ± 0.1	14-21	17.9 ± 0.1	15-19	17.7 ± 0.1	14-21

with the inclusion of young males in the breeding season samples, the mean weight of the perineal glands (813 mg $\pm$ 65.2) comprised about 0.8% of the mean body weight. This percentage of perineal gland weight to total body weight was as high as 1.4% in the largest males.

To gain insight into the seasonal variability of the perineal gland a regression analysis was run using the perineal gland weight as the dependent variable. The initial analysis showed that 93.19% ($P < 0.0001$) of the perineal gland variability could be explained by the other independent variables, the testes weight, seminal vesicle weight, body length (total body length minus tail length), body weight, hindfoot length and ear length. However, of these variables only the testes weight ($P < 0.0001$), seminal vesicle weight ($P < 0.0001$), and body weight ($P = 0.0354$) were significant. Body, hindfoot and ear lengths did not contribute significantly to explaining the variation between seasons. When a stepwise regression was run, the testes weight, seminal vesicle weight and body weight independent variables met the significance level for entry into the model. Of these, however, the body weight was determined to be not significant and was dropped from the model. Thus, this analysis confirmed that the perineal gland and the reproductive organs (testes and seminal vesicles) exhibit seasonal changes whereas the other body weights and measurements do not. The analysis of variance with stepwise regression having an $r^2 = 0.9272$ ($P < 0.0001$) indicates the close association of these three organs, the predictive model for which is:

Perineal gland weight = -18.67 + 0.443 (testes weight) + 0.398 (seminal vesicle weight).

Correlation of the Perineal Gland to Body Size and Reproduction

In order to accurately assess the seasonal relationships of the perineal glands, testes and seminal vesicles including in their relationships to other body measurements, Pearson's Correlation Coefficients were run. Perineal gland weights obtained from samples collected throughout the year were compared with body measurements and weight of the testes and seminal vesicles (Table 2). Not surprisingly, the strongest correlations were between the perineal gland and the two reproductive tissues, the testes and seminal vesicles. The testes, producer of sex steroids, showed a positive correlation with perineal gland weight ($r = 0.933$, $P < 0.0001$). The seminal vesicles, which are secondary sex tissues responsive to the sex steroids produced by the testes, also show positive correlation to the perineal gland ($r = 0.930$, $P < 0.0001$). Correlations of these reproductive organs with body weight and body length (total length minus the tail length) also yielded significant, but lower, correlations ($r > 0.50$; $P < 0.0001$). By comparison, correlations between the reproductive organs and either hindfoot or ear length (< 0.25) were much lower. The relationship of the testes, seminal vesicles and perineal gland weights to the hindfoot length and the seminal vesicle weight to ear length were significant at $P < 0.05$. However, the correlation between the testes and perineal gland weights and the ear length was not significant ($P > 0.05$).

Because the changes in the size and weight of the perineal gland corresponded strongly with the normal breeding cycle for *Sigmodon hispidus*, changes in the perineal gland weight were compared to changes in the reproductive tissue weight throughout the year. As shown in Figure 6, not only do the mean monthly weights of the perineal gland change dramatically (6

Table 2 Pearson's Correlation Coefficients for weights of perineal gland, testes, and seminal vesicles and other body measurements for adult male cotton rats (>50 gm) collected at monthly intervals throughout the year. Sample size = 129. (Definitions and units are the same as in the legend to Table 1.)

VARIABLES	Perineal gland weight	Testes weight	Seminal vesicle weight	Body length	Body weight	Hindfoot length	Ear length
Perineal gland weight	-	0.933	0.930	0.617	0.534	0.192	0.136
Testes weight	0.0001	-	0.888	0.623	0.518	0.230	0.155
Seminal vesicle weight	0.0001	0.0001	-	0.575	0.508	0.184	0.174
Body length	0.0001	0.0001	0.0001	-	0.853	0.456	0.444
Body weight	0.0001	0.0001	0.0001	0.0001	-	0.492	0.527
Hindfoot length	0.0293	0.0088	0.0366	0.0001	0.0001	-	0.409
Ear length	NS	NS	0.0481	0.0001	0.0001	0.0001	-

r

P

A), but the weights of the testes and seminal vesicles go through similar cyclic changes (6 B & C). The increase in weights for these three organs began in February. However, the increase in testes weight from January to February was much more dramatic (a 13-fold increase in weight) than the weight increase from February to March (a 1.8-fold increase). The mean weight changes for both perineal gland and seminal vesicles from January to February were also large, 163-fold and 15-fold, respectively. (The enormous size of this change is due to the extremely small, almost indiscernible size of these atrophied organs during January.) However, unlike the weight increases in the testes, which drop to a 1- to 2-fold monthly increase after February, the perineal gland and seminal vesicles continue rapid increases in weight through March with 5- and 7-fold increases in weight, respectively. By April the changes occurring in these two glands have moderated and fall in line with the smaller increases seen in the testes weight.

Perineal gland, testes and seminal vesicles weights all decreased in mean values during July. Based on the minimal weight requirement for inclusion of an animal in the study (> 50g), the transient decrease in these weights in July is most likely due to the recruitment of spring-born males into the population. This assumption is substantiated by comparison of the mean monthly body weight for animals captured in July, 80.8 g, with the mean body weights for animals captured during the other breeding season months and observing that the July mean is considerably lower. During August and September the mean organ weights rebounded to their previous high levels (with body weight also increasing to 87.5 g and 108 g, respectively), but then a rapid reduction in weight and size occurred in October. Though the testes weight decreased in October, further testicular regression continued so that from November to January

testes weights of all males ranged from 30-50 mg. All three organs remained low in weight throughout the winter until the following February, when the cycle began anew.

Histology of the Perineal Gland

The following photographs are taken from a cranial aspect of the perineal gland. These cross-sections of the perineal region are taken from the craniad third of the segment near the point of attachment of the gland to the penis. At low magnification (Fig. 7), the rectum (1), two lateral (perineal) glandular regions (2) with their muscular investment located lateral to the rectum, the dorsally located urethra (3) and two additional structures lateral to the urethra are clearly seen. The glandular tissue shows bilateral symmetry, with portions lying both lateral or dorso-lateral to the rectum and ventral to the urethra. The glandular areas contain smaller tubules which coalesce to form large tubules or ducts. These large tubules are within a neck-like projection of the gland which connects with the urethra. Two additional structures, which flank the urethra, contain ductal fibrous connective tissue with squamous epithelium and some vasculature. These appear to provide structural support for the perineal gland. The region around the gland is highly vascularized with large regions of extravascular space and sinusoidal areas interspersed around the gland. Although the perineal gland and the rectum are located close to one another, there are no connections between them.

At slightly higher magnification (Fig. 8) the gland appears to have three different regions with a prominent smooth muscle investment surrounding the entire gland. The bulbous portion of the gland contains acini, connective tissue and small tubules. The neck-like projection of the

Figure 7 Cross-section of the perineal region, 11.9 x magnification, of a sexually mature male cotton rat shown from a cranial aspect. 1) rectum 2A) and 2B) perineal gland 3) urethra 4) additional structures. A, B and C indicate areas shown in greater magnification in Figures 8-10.

Figure 8 Cross-section of the perineal region, 31 x magnification (enlargement of Region A from Figure 7), of a sexually mature male cotton rat shown from a cranial aspect. Note the extension of glandular tissue toward the urethra.

FIG. 7

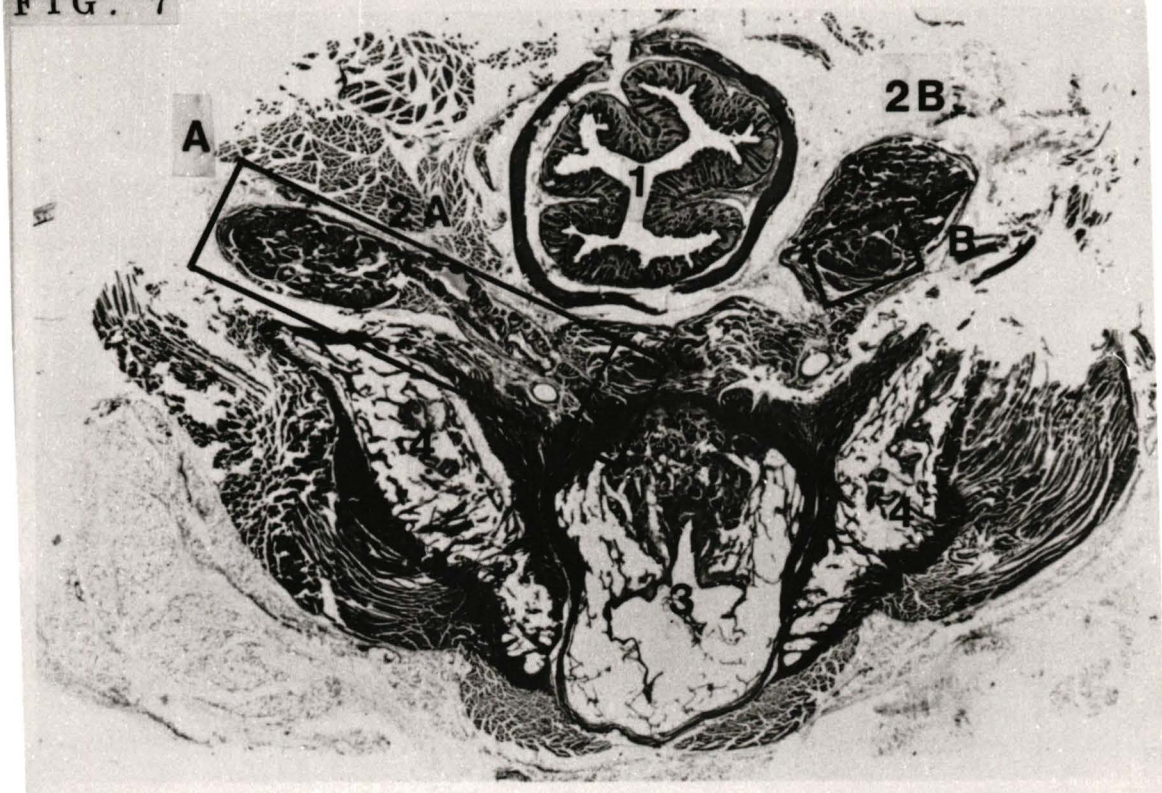
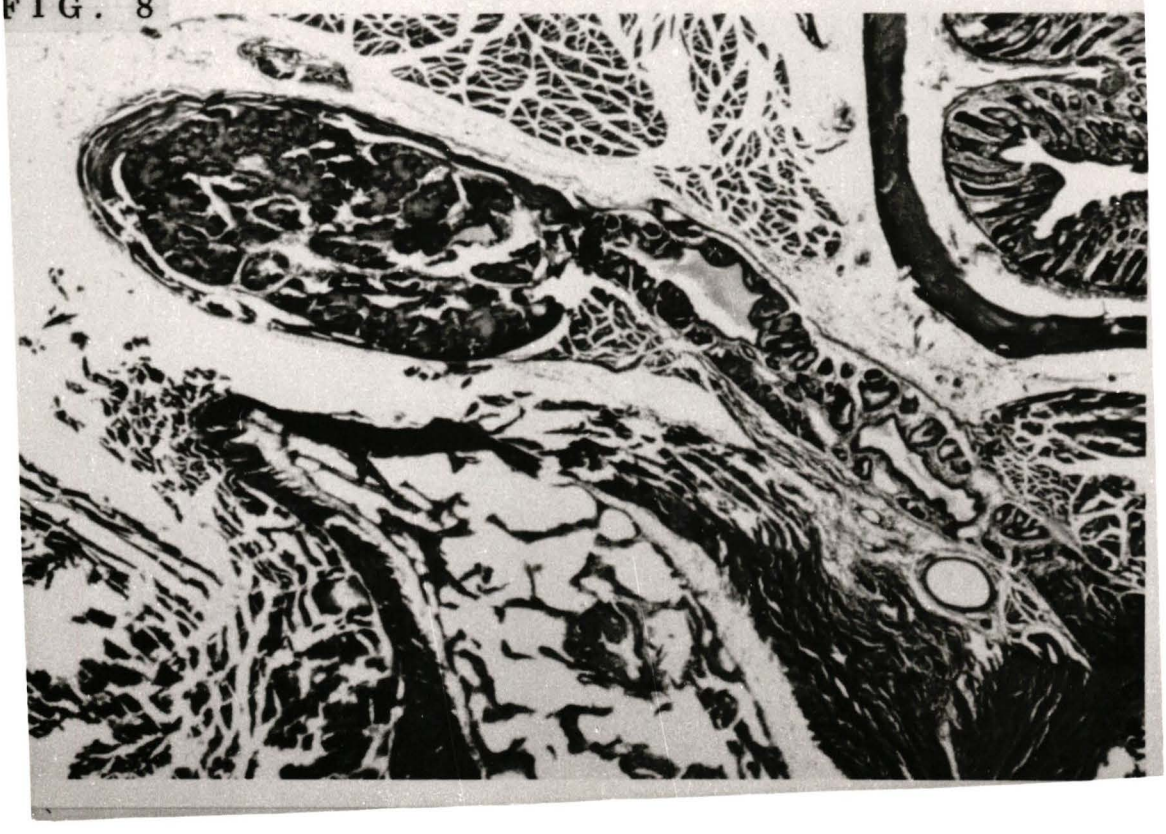


FIG. 8



gland contains larger tubules or ducts and can be seen extending toward the penis. Finally, the head-like ending of the gland abuts the smooth muscle which surrounds the penis. Although the tubules of the neck-like projection are prominent in this cross section, a definitive continuation of these tubules through the head of the gland or the muscle surrounding the penis cannot be visualized. Secretory cells can be seen projecting into the tubular portion of the gland. H & E staining revealed tubules filled with a pink-staining mucinous material which is similar in texture and consistency to thyroid colloid. The material was further characterized using a PAS hematoxylin stain, which stained brightly pink, similar to glycoproteins or glycolipids.

At high power (Fig. 9), the tissue is seen as a multi-lobular, compound tubuloacinar gland with several distinct bundles separated by septa or connective tissues. These individual lobules are surrounded by fibrous connective tissue and the entire group of lobules is surrounded by smooth muscle. Tubules within and between the lobules are lined with epithelium which progresses from a columnar to a more cuboidal shape. The non-tubular regions reveal the mucin-filled acini resembling alveoli. Each individual acinar cell has a large granular cytoplasm and a flattened nucleus located near the basal lamina. Overall, the acinar cells are similar in appearance to the acini of salivary gland tissue.

At the head-like junction of the perineal gland with the penis (Fig. 10), the gland contains reduced amounts of acinar cells with more connective tissue and glandular tissue such as that found in the more lobular portions of the gland. However, as this head abuts the penis the nature of the glandular tissue within the muscular wall of the penis is different from tissue outside of the muscular wall. Within this area the glandular tissue consists of densely staining columnar tissue with larger nuclei and little cytoplasm when compared to the glandular tissue outside of

Figure 9 Cross-section of the perineal region, 112.5 x magnification (enlargement of Region B from Figure 7), of a sexually mature male cotton rat shown from a cranial aspect focusing on one of the septated secretory bundles.

Figure 10 Cross-section of the perineal region, 122.5 x magnification (enlargement of region C from Figure 7), of a sexually mature male cotton rat shown from a cranial aspect focusing on the region where the perineal gland abuts the urethra.

FIG. 9

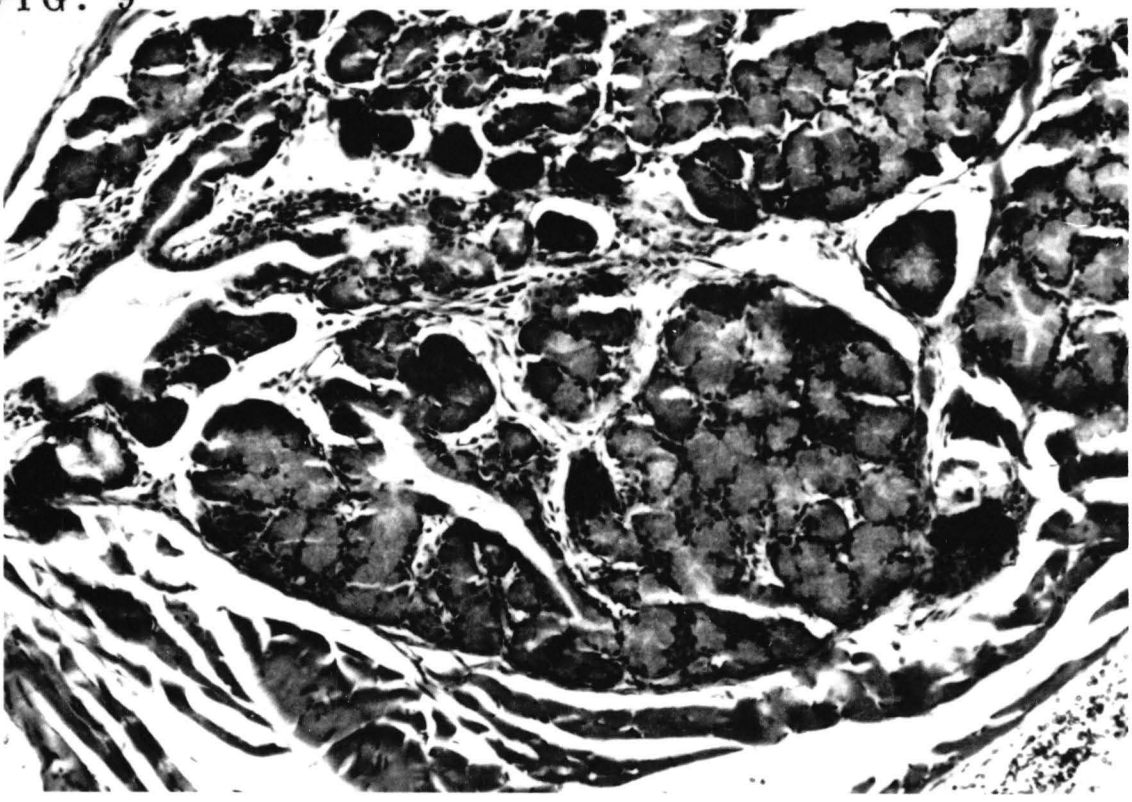
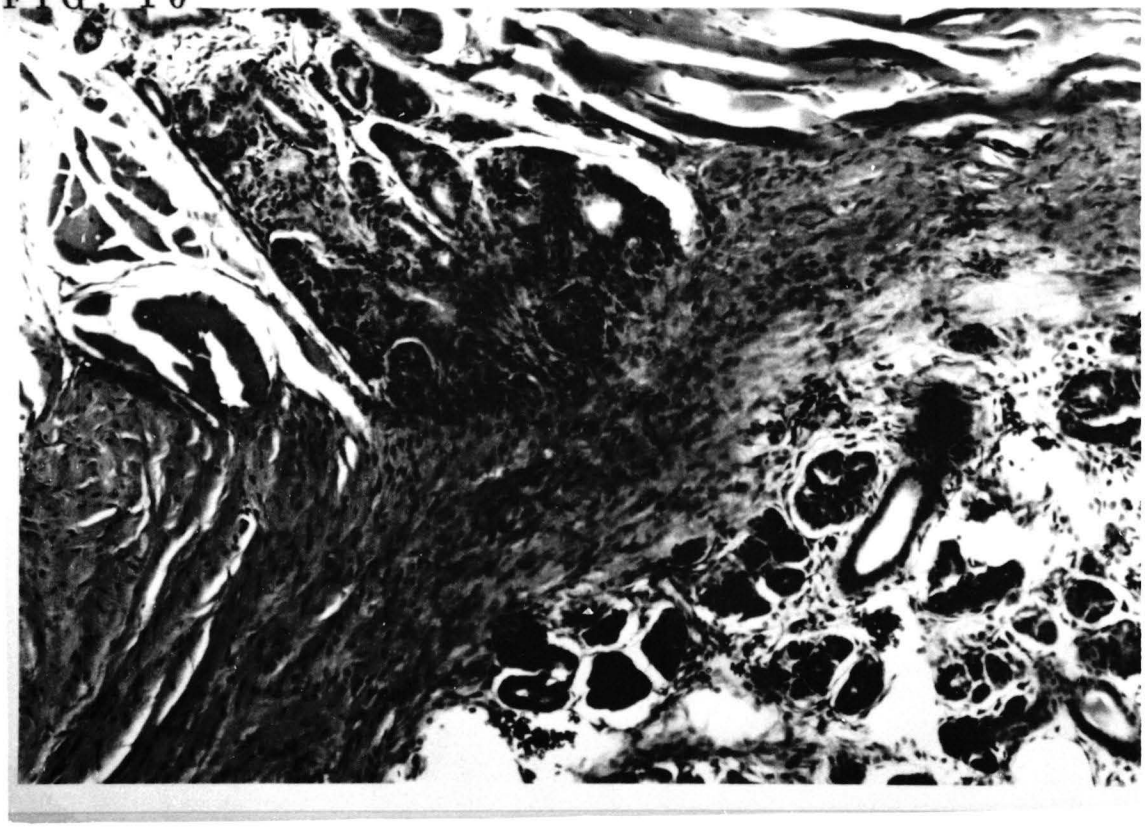


FIG. 10



the muscle. These glandular areas are also surrounded by blood-filled sinusoids. This type of tissue extends cranially up the dorsal portion of the penis and can also be seen in low-magnification cross sections from the second more cranial block of tissue in the same plane as the vas deferens (photograph not shown).

The Effect of the Perineal Gland Homogenate on Trappability

The community of small mammals on a 38 m x 45.6 m grid was determined by capture-and-release methods to contain 28 cotton rats, 27 house mice *(Mus musculus)* and approximately three white-footed mice *(Peromyscus leucopus)* (the latter not ear-tagged) during November 1987. As with any open field trapping study the identification of captured animals can only yield an approximation of the total population. Constant recruitment, immigration and emigration of individuals and trap resistance among certain individuals make it impossible to determine exact population size and structure. The timing of the study occurred at the latter part of the breeding season when there is reduced sexual activity and animals are preparing for the reproductively dormant winter months. The sex ratios on the grid were skewed slightly toward males (17:11 in *Sigmodon* and 16:11 in *Mus*), but the populations appeared to be healthy and growing as noted by the presence of several young adults. This sex-ratio difference is consistent with the larger number of male cotton rats caught during the 300 trap night test period (total number of male cotton rat captures = 30; total number of female cotton rat captures = 20). However, it would

not influence the comparison of trappability in the homogenate treatment group versus the control group because these comparisons were made for animals sorted by sex.

The presence of the perineal gland homogenate had a significant effect on the trappability of male cotton rats at the beginning of the non-breeding season. In the study, there were 15 each of treatment and control traps. Of 30 male captures, 86% (or 26 captures) were caught in homogenate-baited traps. This is significantly different from an expected random trapping pattern ($X^2=16.14$; d.f.$=1$; $P<0.001$). Additionally, the mean weight of males caught in homogenate-baited traps (54.17 g) was significantly greater (t-test, $P< 0.01$) than the mean weight of control animals (42.5 g) (See Table 3).

However, the homogenate from the perineal gland did not elicit an interpretable result in the females trapped on the grid. Nine female captures were in the homogenate-baited traps, whereas eleven captures were in the control traps. This was not significantly different from an expected random trapping pattern ($X^2=0.2$, $P>0.90$). Also, in examining the mean weights of the two groups by Student's t-test, there was no significant difference between the females caught in traps baited with homogenate from the perineal gland and the controls. (See Table 3).

In the second trapping study, late in the breeding season in September 1988, the tagged population consisted of 42 male and 31 female cotton rats and 16 male and 12 female meadow voles. In addition, a few house mice, harvest mice *(Reithrodontomys humulis)* and marsh rice rats *(Oryzomys palustris)* were captured but not tagged. During the 900 trap-night study, 327 captures were made for a 36.3% trappability. The study used six scent baits: 1) a muscle homogenate (control), 2) a perineal gland homogenate, 3) the chloroform fraction from a muscle extraction (control), 4) the chloroform fraction from a perineal gland extraction, 5) the methanol

Table 3 Effect of the perineal gland homogenate on trappability in the first field study. There were equal numbers of traps with control and homogenate-laden baits.

		BAIT	
		Control	Homogenate
Sigmodon hispidus males	Number trapped	4	26
	Mean weight in g	42.5	54.2
Sigmodon hispidus females	Number trapped	11	9
	Mean weight in g	68.6	56.5
Mus musculus males	Number trapped	6	12
Mus musculus females	Number trapped	8	9

fraction from the muscle extraction (control), and 6) the methanol fraction from the perineal gland extraction.

When the total *Sigmodon* population was tested against a random trapping pattern using a Chi-square test, there was no significant difference from random. However, when the *Sigmodon* population was grouped according to sex, the males did not exhibit a random response to the scents placed in the traps ($X^2=12.86$; d.f.$=5$; $P<0.05$). Instead, there were fewer captures associated with the methanol/water fraction from the muscle homogenate extract (9 of 118) and a larger than expected number of captures in the traps baited with muscle homogenate. Further evaluation of the male captures showed that when divided into either sexually active or inactive animals, neither group showed significant differences from random ($X^2=7.4$ and 4.4, respectively; d.f.$=5$; N.S.). The female cotton rats responded randomly, regardless of their reproductive status (See Table 4). Additional Chi-square tests on the trap responses of *Microtus pennsylvanicus* in the same study showed no variation from an expected random trapping pattern for the total population or for either sex. (See Table 5).

Table 4 Numbers, weights and sexual status of *Sigmodon hispidus* caught in a 300 trap-night study using extraction and homogenate baits. Scrotal/abdominal refers to the position of the testes; open/closed refers to the status of the vaginal opening. Animals showing ambiguous sexual characteristics were dropped from the study.

	Polar muscle extract	Non-polar muscle extract	Muscle homogenate	Polar perineal gland extract	Non-polar perineal gland extract	Perineal gland homogenate
Total animals	24	33	42	32	36	29
X wt (g)	67.5	57.7	72.5	69.0	68.5	69.9
Total males	16	22	30	22	16	18
X wt (g)	71.8	59.5	74.1	69.9	69.1	66.2
Scrotal males	6	7	16	13	8	10
X wt (g)	86.5	73.4	89.2	79.8	83.4	93.2
Abdominal males	10	15	14	9	8	8
X wt (g)	62.9	52.9	56.9	53.1	54.8	32.5
Total females	8	11	12	10	20	11
X wt (g)	67.6	70.0	67.0	73.5	71.3	74.0
Open females	3	6	6	5	12	4
X wt (g)	76.0	85.3	84.0	91.8	93.9	76.2
Closed females	5	5	7	5	8	7
X wt (g)	57.4	51.5	55.4	57.4	30.3	75.0

Table 5 Numbers, weights and sexual status of *Microtus pennsylvanicus* caught in a 300 trap-night study using extraction and homogenate baits. Scrotal/abdominal refers to the position of the testes; open/closed refers to the status of the vaginal opening. Animals showing ambiguous sexual characteristics were dropped from the study.

	Polar muscle extract	Non-polar muscle extract	Muscle homogenate	Polar perineal gland extract	Non-polar perineal gland extract	Perineal gland homogenate
Total animals	14	14	19	17	14	6
X wt (g)	46.3	45.6	44.8	46.8	42.2	49.2
Total males	10	8	9	13	8	6
X wt (g)	50.8	47.1	57.1	49.5	46.4	49.2
Scrotal males	10	8	9	13	8	5
X wt (g)	50.8	47.1	57.1	49.5	46.4	49.4
Abdominal males	0	0	0	0	0	1
X wt (g)	0	0	0	0	0	48.0
Total females	4	6	10	4	6	0
X wt (g)	46.5	43.7	38.1	37.8	36.7	0
Open females	3	5	6	2	5	0
X wt (g)	46.7	42.2	41.3	38.5	37.6	0
Closed females	1	1	4	1	1	0
X wt (g)	46.0	51.0	33.4	38.0	32.0	0

DISCUSSION

In order to determine the anatomy, nature and function of this previously undescribed perineal gland complex in *Sigmodon hispidus,* several aspects of this remarkable organ were examined. Studies of both the gross anatomy and histology of the gland complex were performed to define the physical structure of the organ and to determine any points of connection with other organs or structures which could serve as delivery systems for glandular products. The seasonality of the gland was examined through statistical evaluations of samples collected on a monthly basis throughout the year. The correlations of these seasonal variations to standard physical measurements and to changes that occur in other organs were also evaluated. Finally studies were conducted to assess the function of the perineal gland products and their effects on natural cotton rat populations.

Gross Anatomy - Location and Description of the Perineal Gland

The subcutaneous location and close association of the perineal gland with the rectum and penis indicated three possible routes of delivery for any glandular products. Many microtine rodent species possess enlarged sebaceous glands which extrude their products onto specialized hair or skin pads, as in the caudal gland of *Dicrostonyx groenlandicus* (Quay, 1968). In

Sigmodon, however, the large size of the gland complex, the presence of only loose fascial attachments to the skin and the lack of specialization of either the skin or hair texture did not suggest delivery through the skin. In addition, the loose fascial attachments to the rectum made a fecal delivery (as occurs in *Microtus agrestis* [Khan, 1984]) doubtful. This left the third possible route as the most plausible one for the delivery of glandular products, i.e., delivery through the urinary tract via a connection with the urethra, as seen in beaver (Svendsen, 1978). Dissection revealed extremely strong ligamentous attachments of the gland to the penis, further supporting the hypothesis of a close association of the perineal gland to the reproductive and lower urinary tracts, but no duct connecting the gland to the urethra could be observed from a gross examination. Histological studies which will be discussed later, however, confirmed this urinary route.

Seasonality of the Perineal Gland

The seasonal changes in the perineal gland begin with its hypertrophy at the onset of the breeding season and proceed through regression of the gland at the end of the breeding season. The increase and subsequent decrease in mean weight corresponds closely with the seasonal weight changes of the reproductive tract organs (Fig. 6). As with the dramatic changes in sizes and masses of the testes and seminal vesicles, the perineal gland enlarges from an indiscernible organ that cannot be dissected away from the surrounding tissue to a large, fleshy tissue whose mass composes up to 1.4% of the total body mass in some individuals. This is comparable with the percent of total mass contributed by the testes (up to 1.8%) and the seminal vesicles (up to

1.6%) observed during the breeding season in this study. When considered as a group, these organs can comprise up to 4.8% of the total body mass and would represent a substantial energy maintenance cost. The association of the perineal gland cycle of regression and recrudescence with the seasonal cycling of the urogenital system was further illustrated by the analysis of variance with stepwise regression. This analysis indicated the extremely high degree to which the variability of the perineal gland may be explained by the variations of the testes and seminal vesicles ($r^2 = 0.9272$; $P < 0.0001$).

The highly significant variability of testes, seminal vesicle and perineal gland weights as well as the similarities in pattern of growth and regression strengthens the evidence of a link between the perineal gland and the reproductive functions in male cotton rats. Because seasonal regression of the sex organs is used as an energy-saving device in small mammals to improve winter survivability (Vaughan, 1986), the similar regression of the perineal gland could also serve to reduce winter energy requirements. With the combined mass of these three organs comprising up to 4.8% of the total mass of the animal the energy savings during organ regression would be considerable. However, this reduction and subsequent energy savings can only be achieved in the perineal gland because, as with the testes and seminal vesicles, the functions or products of the perineal gland are no longer needed during the non-breeding winter months. This further indicates that the perineal gland serves the reproductive system in some capacity, perhaps as a secondary sex organ.

A closer look at the cyclicity of these three organs shows similarities that could only occur if these systems were under simultaneous control. When comparing the monthly mean weights of the perineal gland, testes and seminal vesicles a definite pattern was seen (Fig. 6).

The mean weight of the testes at the onset of the breeding season made a more dramatic increase than the increases in weights of either the perineal gland or seminal vesicles. Conversely, at the end of the breeding season (October) the weights of the perineal gland and seminal vesicle plunged to their non-breeding, winter masses whereas the testes decreased in weight more gradually throughout the fall months. The same pattern of a rapid reduction in weight of the seminal vesicles while the testes weights continued to decline over a longer time was also seen in the Beer and Meyer (1951) study of endocrine organs in muskrats. Since the status of the seminal vesicles has been shown to be under androgen control in other rodents (Tamarkin et al., 1976) it follows that their hypertrophy would begin after the testicular enlargement with its subsequent increase in androgen production. Additionally, the reduced androgen production which precedes a reduction in testes mass causes the rapid decrease in seminal vesicle mass that occurs. Because the changes in the mass of the perineal gland occur in synchrony with those of the seminal vesicles, it can be assumed that the changes are under the same androgen control that regulates the seminal vesicle growth and regression. Additionally, androgen control of specialized sebaceous glands has been clearly illustrated in several families of mammals through castration/androgen replacement studies. For example, glandular reduction by castration and growth or maintenance by castration combined with androgen replacement has been demonstrated in the anal and chin glands of rabbits (Mykytowycz, 1965), in the proctodeal gland of the short-tailed vole (Khan, 1984), in the supracaudal gland of the guinea pig (Martan, 1962) and in the ventral gland of the Mongolian gerbil (Thiessen, et al., 1968). In addition, Thiessen, et al. (1968) showed that not only is the glandular size of the midventral sebaceous gland of the Mongolian gerbil under androgen control, but the frequency of territorial marking with glandular

products is also controlled by systemic androgen levels. It is, therefore, logical to assume that the perineal gland in *S. hispidus* is under androgen control as well. The androgen control may extend not only to the size of the organ, but also to the nature and volume of organ secretions and the placement of those secretions within the environment.

The only deviation from this seasonal increase and then decrease during the breeding season occurs as an interesting dip in the mean weights of all three organs during July (Fig. 6). Quay (1953) observed these same patterns in the kangaroo rat *Dipodomys merriami*, a species with sexual dimorphism in the dorsal skin gland. Initially, males exhibit enlargement of the gland, after which moderate percentages of adults possess actively secreting glands during their breeding season (February through June); the size and activity of the dorsal gland tapers off through September as the breeding season gradually closes. As with the July dip in *S. hispidus*, the mean gland area shows a drop in May with a rebound in June. Quay made no remarks on this drop; however, the decrease in mean weight during July in *S. hispidus* and May in *D. merriami* corresponds to the time at which spring-born animals are first entering the population of reproductive animals in both species. This introduction of young, small males which are currently undergoing their sexual maturation would tend to decrease the mean weights for the month as was the case in this study. However, because mean weights were not provided in the study by Quay (1953) this can only be assumed from the reproductive information given, which stated that young of the year (already two-thirds grown) appeared as early as April 8.

This explanation of the July dip in *S. hispidus* is corroborated by the high standard errors for the reproductive organ weights of July males, which indicates the great variability within the population at that time. In addition, when the records for individual animals are examined from

the pool of July animals, two groups stand out within the population. A scattergram of the raw data for July which compares the testes and seminal vesicles weights to the perineal gland weights shows the distinct populations (see Appendix B). Animals with smaller length and mass often have reduced mass of the reproductive organs and perineal gland and may be lacking marked convolutions of the epididymis (which indicate infertility). This would indicate that these animals are just beginning their reproductive lives. The second group consists of larger males with completely mature sexual organs with convoluted epididymides (and hence fertility). These two groups within the population are probably comprised of larger males which overwintered (perhaps late summer- or fall-born animals) and have been reproductively active since the onset of the spring breeding and the second wave of spring-born males just entering the reproductive population. Therefore, the July decreases in mean organ weights are not due to real reductions in organ weights of individuals but are explained by the changing composition of the population as young-of-the-year males enter the breeding population.

Correlation of the Perineal Gland to Body Size and Reproduction

Pearson's Correlation Coefficients were performed on the raw data to statistically confirm the observed pattern of seasonal change and the relationship of the perineal gland to the testes, seminal vesicles and other standard measures (Table 2). The extremely high correlations of the perineal gland weights to those of the testes ($r = 0.933$) and seminal vesicles ($r = 0.930$) indicate that a high probability exists for the gland to be associated with the reproductive system. In addition, there appears to be a trend for all three organs to have slightly higher correlations

to body length than to body weight. This contrasts with the information obtained from the regression analysis with stepwise progression which, indicated that the body length has no significant role in explaining the variation seen in the perineal gland weight. McCravy and Rose (1992) also determined that the body length had consistently lower r-values than body weight when using logistic multiple regression analysis for predicting reproductive status. However, this seeming contradiction might be explainable. Young, reproductively active males which devote large amounts of energy for reproduction cannot accumulate as much body mass as a nonreproductive animal. Thus although they achieve sexual maturation and their adult length, they do not accrue additional bulk or fat until after the rigors of the breeding season are over. In addition, weight reductions in older, heavier males have been observed during the breeding season (without apparent adverse effects on their survival or reproductive readiness since they continue to be trapped and they maintain enlarged, scrotal testes) as these animals convert body fat for energy to be used in reproduction. Because these lighter weights do not adversely affect the reproductive capacity of the animals (as judged by the presence of marked convolutions of the epididymis), these males remain in the reproductive population with diminished weights yet the same body lengths. The variability in the weights of reproductive males therefore is larger than the variability in body lengths and this results in lower correlations with the reproductive organs. So, although this variability gives body weight a greater predictive value for reproductive status, higher correlations are found between the body length (which appears to be a less variable indicator of maturation) and the organs associated with reproduction.

Histology of the Perineal Gland

Completion of the gross anatomy studies prompted a histologic examination in order to determine the nature of the tissue and to define any attachment points or close associations of the perineal gland with other nearby organs or systems. Initial attempts to study fully enlarged glands were thwarted by the extremely large size and oily nature of the gland. Because effective dehydration could not be achieved on these large pieces of tissue, smaller tissues from only partially hypertrophied glands were studied. Thin sectioning showed conclusively that in its rudimentary state the perineal gland is physically connected to the penis via a tubular extension which penetrates the muscle surrounding the penis (Fig. 7). In addition to establishing a direct link with the reproductive tract, the histologic exam also verified the glandular nature of the tissue. Figures 8 through 10 show the compound tubuloacinar nature of the gland through differential staining with H & E. Additional PAS staining of other sections revealed bright pink staining of the contents within the tubular portion of the gland. This pink coloration indicates that the material is either glycoprotein or glycolipid in nature.

This compound tubuloacinar structure of the gland bears a strong resemblance to the flank glands of certain microtine rodents. The most similar glands to the *S. hispidus* perineal gland are the highly developed and highly specialized microtine sebaceous glands which are observed as large, compact subdermal masses occurring in *Arvicola* and certain species of *Microtus*. These structures occur in both sexes of all species of *Arvicola* and are present in several species in the subgenus *Aulacomys* of *Microtus* (Quay, 1968). *Microtus gregalis* exhibits the gland in adult males, but not in immatures or females, much like the pattern seen in the perineal gland of *S. hispidus*. In *M. leucurus* the gland is highly developed in adult males during the summer and rarely seen in females; this follows the seasonal implications seen in the cotton

rat. In *M. richardsoni* the gland is present in all ages of both sexes. These similarities in seasonal and sex variations along with the similar massive, complexly branched, multi-lobulated tissue observed histologically in these species parallel the findings of the histologic studies in *S. hispidus*. In addition, Quay's findings that the glandular products of the flank gland are primarily lipid secretory material support the assumptions that the perineal gland secretion may be glycolipid in nature.

Although there are several similarities between the perineal gland and the flank glands in *Arvicola* and some species of *Microtus*, a major difference lies in their methods of delivery of the secretory product. In the flank glands the secretion is excreted via hair follicles of relatively simple structure; however, the histologic studies of *S. hispidus* indicate a urinary tract delivery. To understand the delivery route for glandular products, examination of Figures 7, 8 and 10 shows the location of the gland in the perineal region and its extension through the muscular band which surrounds the penis. The central lumen of this extension is filled with the mucinous glycoprotein or glycolipid product of the acinar cells located in the lobular portion of the gland. The intrusion of the neck and head of the gland through the muscle into the erectile tissue of the corpus cavernosum provides direct delivery of the glandular product into the root of the penis. Once beyond the muscular wall of the penis, transport or diffusion of the glandular secretion into the urethra would occur for ultimate delivery with the urine. The exact method for the product delivery into the urine is uncertain because histologic sections did not reveal a direct ductal entrance into the urethra. Either a direct opening into the urethra or active or passive diffusion of the product through the membrane of the urethra may occur. A direct opening from the gland into the urethra as is seen in the castor gland of the beaver (Svendsen,

1978) may exist, but was not observed in this study. However, portions of tubules containing the mucinous secretory product were seen within the muscular band of the penis (photograph not shown) suggesting that a ductal route into the urethra. If this is the case either passive washing of the product from the gland (as in the castor gland) or active propulsion of the product into the urethra may occur. Because the entire gland is surrounded by a prominent investment of smooth muscle, the gland seemingly is able to propel its product into the penis through muscular contraction. If this pulse passes through a direct opening to the urethra, specialized marking behaviors, conditions and stimuli may exist. If the delivery from the interior of the root of the penis occurs through a more passive diffusion into the urethra, marking may occur with all urinary delivery during the breeding season. However, controlled delivery may still occur without a direct urethral opening if active transport through the urethra takes place.

To gain more insight into the precise nature of the delivery of the product, more extensive histologic examinations, behavioral observations and observations of the effect of the glandular product on conspecifics and other species were done. Although behavioral experimentation is difficult because of the dense structure of cotton rat habitat and the vagile, dispersing nature of this species, further studies were done in an attempt to define the effects of the glandular secretion.

The Effect of the Perineal Gland Products on Trappability

Although laboratory studies provided an understanding of the gross location, the attachment to the urogenital tract (as seen on histologic examination), and the seasonal variations of the perineal gland, they did not give an indication as to the function of the gland. Therefore, two field trapping experiments were designed to evaluate the information content of the perineal gland and its products on populations of cotton rats and secondarily on other mammals in the community. The first field study was run at the close of the breeding season, November 20th through 29th, using homogenized perineal glandular tissue (from adult males collected in the breeding season) versus solvent control. The results showed a significant preference for traps baited with the extract among male cotton rats, but no significant preferences with females (Table 3). These results apparently contradict an earlier study on the effects of scents on trapping done by Summerlin and Wolfe (1973). Using clean cotton for control traps and traps scented with cotton previously used as cotton rat bedding in treatment traps they found an overall preference for clean traps in a cotton rat field trapping study. When grouped by sex and reproductive status, reproductive males showed no trapping preference, nonreproductive males and females preferred clean traps and reproductive females preferred scented traps (Summerlin and Wolfe, 1973).

Differences between the Summerlin and Wolfe study and this first field study could have been caused by the differences in the reproductive status of the populations. The Summerlin and Wolfe sampling was conducted throughout the spring and summer and therefore gave an indication of the preferences exhibited by animals in the reproductively active season. Thus, the investigators were able to relate the individual's reproductive status to the response to the scents emanating from the used bedding. The late November timing of my field study, however,

precluded drawing any conclusions on the effect of the presence of the perineal gland homogenate on reproductively active populations since the breeding season was coming to a close (as exhibited by the preponderance of males with abdominal testes). Therefore, the trapping preferences that were observed in this study can be indicative of non-breeding season response to the homogenate. Further studies would be necessary to determine the effect of the homogenate on trapping success during the breeding season.

Additionally, because the Summerlin and Wolfe (1973) study used bedding as its scent treatment where as my study used homogenized tissue from the perineal gland, further differences in responses could be due to these bait differences. While the scents of the perineal gland homogenates were derived from glands of adult males in the breeding season, the used bedding may have contained a number of scents that could have contributed to a response. Fecal, urinary, sebaceous and salivary secretions all might have been present in the used bedding, and as such the trapping responses in the various categories of animals may have been to any one or a combination of these signals. Because the sex and reproductive status of the animal or animals previously housed in the bedding used as bait was not indicated, it is difficult to determine the nature of the change seen in the trapping response. We do not know whether the aversion/attraction was to male or female scents, to scents associated with a particular phase of reproduction (estrus/anestrus, reproductive or nonreproductive) or to members of a particular social class (dominant/subordinate).

Because females were neither repelled nor attracted to the extract in the trap (control traps caught 11 animals; treatment nine), there was no apparent effect of the perineal gland product on females which were slowing in their reproductive activity. Although the lack of

reproductive males in the population indicated that the breeding season was at a close, the condition of the females on the grid indicated that, though no longer receptive to breeding, some animals on the grid were still lactating. This may have influenced the trapping somewhat by attracting females with the higher energy requirements of lactation to the grain bait. While this may have increased the potential for trapping, it would not cause the females to prefer either treatment or control traps. Ultimately, the random trapping response of the females indicated that not only did the homogenate bait have no effect on the females, but the filter paper wedges that were saturated with solvent were adequate controls for the study since randomness was achieved. Although the female cotton rats showed no preference in this initial study, no conclusions can be drawn at this time on the effect of the perineal gland products on breeding females. For more conclusive results on the effect of the perineal gland on female cotton rats, additional field studies need to be done during the breeding season.

The significantly high percentage (86%) of males trapped in the traps baited with homogenate indicated an attractant effect of the perineal gland or its products on male cotton rats. Additionally, the males caught in traps baited with homogenate had significantly heavier mean body weights than the males from control traps. This indicated that larger males were more responsive to the homogenate than were the smaller animals. These animals were probably part of the breeding population earlier in the year when breeding was taking place and they may be dominant animals within the population due to their large size.

Because territorial marking using scents is widespread among mammals, it is possible that the products of the perineal gland may be placed in the environment by the male as a territorial marker. In this study, response to an unfamiliar scent placed in the environment was shown by

the increased numbers and weights of males trapped in traps baited with homogenate. Male cotton rats, especially large males, may be drawn to the scent in an effort to overmark the scent by covering that scent with their own odor. This behavior has been demonstrated in rabbits (Mykytowycz, 1965), where the dominant, often largest, males are the most frequent markers of territorial boundaries.

Additional laboratory studies on trap response and marking behaviors also indicated different reactions to situations based on the social status of the animal. The Summerlin and Wolfe (1973) study of cotton rats showed that dominant males are most likely to enter an unscented trap before subordinates. The second-ranked male would have the next highest trap frequency and the lowest ranked animals in the social order would have the lowest trap response. Their study further showed that socially naive animals (animals isolated from 10 days of age) showed no trap preference when exposed to both unscented and scented traps, but that naive animals which had been exposed to a socially dominant male and presented with the same trap choices preferred the unscented traps. This experiment demonstrated the use of odors as an indicator of social dominance to a conspecific.

More specific behavioral studies in Taiga voles (Wolff and Johnson, 1979) indicated that dominant males exhibit marking behavior when introduced to a neutral arena with a subordinate male (females seldom exhibit scent marking behavior). Rarely did the subordinate male exhibit a marking response in these instances. Introduction of a dominant male into a subordinate's area also induces the dominant male to overmark the subordinate's scent (subordinate males will not overmark a dominant's scent). These reactions suggest that the marking may serve as a nonaggressive form of dominance or territorial defense. Marking can also be induced in other

instances. Placing a new object into familiar territory will cause the resident animal to mark the unfamiliar object, thereby giving it a familiar scent. In addition, introduction of a female or another adult male will stimulate the adult resident to mark its home area, again indicating the use of scent marking for identification of a home area (Wolff and Johnson, 1979).

The use of scent marking for these functions may help explain the male trapping response seen in this first perineal gland trapping study. Males may be drawn to the scent to investigate the intrusion of an unfamiliar male scent within their territory. In the case of dominant males, the investigation may be preliminary to expressing an overmarking behavior as is seen in the Taiga voles. Subordinate males may be drawn to the odor to determine the identity and status of the foreign animal with respect to their own position within the social order. This seems to contradict the response seen in the Summerlin and Wolfe laboratory and field studies; however, the conditions in both cases varied from the conditions of this study.

In the laboratory study of initial trap response (Summerlin and Wolfe, 1973) the subordinate males were exposed to the traps while in the company of dominant animals, but in the field conditions of my first trapping study it can be assumed that the area of the trapping grid allowed enough space that all animals would have the opportunity for solitary trap investigation. The laboratory conditioning of naive animals would also have skewed their responses to scented traps because the only social scent exposure that they experienced resulted in a negative situation, i.e., meeting dominant males. Had the naive animals been exposed to more varied and normal social situations, their responses may have been different. Because the males in this first field trapping experiment experienced normal socialization from birth, the inquisitive response to a foreign male scent may be the more natural response.

Finally, because the timing of the Summerlin and Wolfe (1973) studies occurred during the breeding season, whereas my first field study occurred during the non-breeding season, a different response may have been elicited. The lack of trap preference in the reproductive males of the breeding season studies may occur because the attention of the males is directed primarily to sexually active females. Additionally, breeding season investigative behavior by subordinate males may occur less often than non-breeding season investigations since natural inquisitiveness could promote encounters with a dominant male, which would exhibit the higher levels of aggression that accompany the breeding season. Once the rigors of the breeding season are over, both dominant and subordinate males may begin to show greater interest in scents within the environment. This might explain the large numbers of males that were trapped in homogenate-baited traps in my first field study.

A second study of response to traps baited with perineal gland products was conducted after homogenization and chemical separation of the homogenate. This study was conducted in September and October near the end of the breeding season. The chemical separation was carried out with the intent to determine the chemical nature of the active component of the glandular secretion. The second experiment compared the attraction/repulsion to six different fractions. Chi-square tests of the trapping results (Table 4 and 5) showed no deviation from a random trapping pattern in the different fractions of the perineal gland. Male cotton rats did show a higher response to muscle homogenates than to the other bait scents. However, when the males were divided into sexually active or inactive groups, the responses were not significantly different from random. This lack of consistency in the statistical patterns and the contradiction with the results from the initial trapping study could indicate that although the male cotton rat

response was not random, it may not be an accurate or conclusive response to the glandular secretions either. Thus, the male response to the perineal gland secretion is difficult to evaluate.

In addition to a questionable male cotton rat response there were no other non-random responses observed in these two natural populations. Examination of the responses of the total cotton rat population, the female cotton rats, the total meadow vole population, male voles and female voles showed no patterns that were significantly different from random. This lack of measurable difference may have been due to a number of factors.

Although in my second field study the perineal gland products showed no responses, the first study showed a dramatic difference in male response within a smaller sample of cotton rats. The results of the first study make it appear that other factors in the second study may have masked or eliminated the response that I anticipated based on the first study. Laboratory processing of the homogenates may have altered one or more chemically active compound, thereby eliminating normal behavioral responses. In the initial study a 70% ETOH solution was used for homogenization and l-ascorbic acid was added to reduce oxidation. In the second study, which involved both homogenization and chemical extraction, the whole tissue was homogenized in water to facilitate the chemical extraction. Although homogenization in water simplified the extraction procedure it may have inactivated the homogenate because a suitable alcohol solvent was not present for dissolving the glandular products. In addition, l-ascorbic acid was not added, so the active compounds may have been oxidized and rendered inactive. Although simplification of the homogenization procedure should have allowed for a clean extraction, it may have prevented any significant response to either fraction of the perineal gland. Because many olfactory chemicals are volatile in nature or possess specific, unique structures which evoke an

olfactory response, it is possible that the methodology for extraction, suspension, drying down or resuspension of the homogenates may have altered or driven off the active component of the perineal gland product.

In addition to the possibility that laboratory processing may have inactivated the glandular secretions, the survival needs of the population may have prevented a positive response to the perineal gland products. The large number of animals found in the study area (101 ear-tagged cotton rats and meadow voles plus a small percentage of untagged animals of other species) may have caused great competition for the grain bait that was present. If the environment had reached its carrying capacity the primary need for survival could override any territorial or reproductive behavioral responses.

Besides the environmental and chemical factors influencing this study the choice of male cotton rat muscle for the control may have been a poor one. Since the leg, flank and hip muscles used are in close proximity to the perineal gland it is possible that scents associated with the perineal gland may be incorporated to a lesser degree in the adjacent muscle. This would confound the field studies by essentially eliminating or reducing the effectiveness of the control.

Conclusions

The perineal gland in *Sigmodon hispidus* is a seasonally cyclic, subcutaneously located organ with a strong attachment to the penis. The seasonality of the gland is strongly correlated with the seasonal changes which occur in the testes and seminal vesicles. These correlations

indicate that, as with the seminal vesicles, the seasonal changes in size, weight and product secretion of the perineal gland are under androgen control.

The gland produces a mucinous secretion similar in texture to thyroid colloid which stains brightly pink (as in glycoproteins or glycolipids) when using PAS hematoxylin stain. This secretion is delivered into the environment via the urinary tract. This delivery route was confirmed by histology, which revealed the projection of glandular tissue through the smooth muscle which surrounds the urethra.

The function of the glandular product in the natural environment is still uncertain. The cyclicity and correlation to primary and secondary sex organs indicate that the gland almost certainly serves some function in the reproductive process. Preliminary testing in field studies suggests that the product may serve as a male territorial marker. However, due to the late fall timing of the first field study, no indication of the effect of the product on actively breeding females could be drawn. Additional studies designed to further explore the chemical nature of the secretion and the effect it produced in natural populations late in the breeding season yielded ambiguous results. Therefore, conclusions about the function in the field cannot be drawn at this time. Hopefully the details reported here will prompt further studies into the production, function, physiology and chemistry of the perineal gland and its products in the hispid cotton rat.

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Appendix A - Raw Data from Necropsied Animals

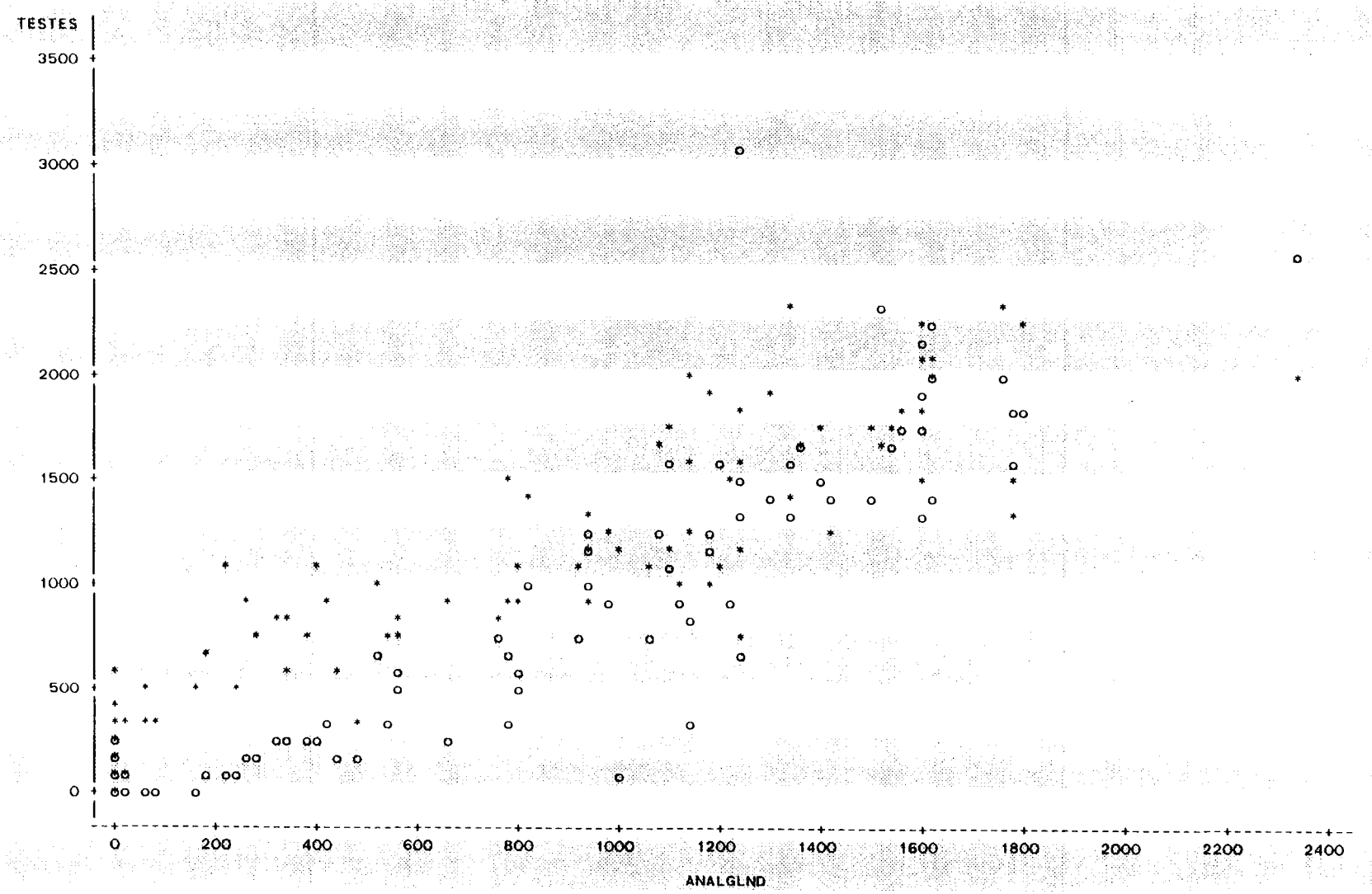
OBS	ANALGLND	TESTES	SEMVES	BODYLNTH	BODYWT	HINDFOOT	EAR	MONTH
1	486	355	125	134	74.2	30	17	9
2	75	360	13	137	78.0	30	17	9
3	1340	2310	1600	145	101.5	32	19	9
4	15	60	1	121	73.4	29	15	9
5	1560	1837	1765	147	117.3	32	17	9
6	784	1514	320	143	95.5	31	17	9
7	1138	2040	827	167	137.0	32	18	9
8	1100	1740	1600	147	97.8	30	18	9
9	1	224	39	123	73.0	29	16	9
10	448	567	164	143	97.7	31	18	9
11	1600	1797	1297	157	144.0	33	18	9
12	1782	1359	1822	159	145.6	30	19	9
13	1229	1532	900	145	100.7	31	16	9
14	1140	1232	303	156	103.7	31	19	9
15	1	60	1	129	72.3	30	16	9
16	1	50	1	123	51.1	29	16	9
17	1	38	2	145	134.0	31	18	9
18	1230	1850	3080	158	113.0	31	19	9
19	1607	2283	1780	167	155.0	30	18	5
20	559	770	466	138	83.0	27	15	6
21	1247	720	670	173	143.0	30	18	5
22	984	1209	950	131	74.9	28	17	6
23	922	1064	784	156	101.2	30	18	5
24	1240	1550	1530	158	110.9	30	18	5
25	1351	1688	1651	168	121.1	28	19	5
26	1546	1717	1647	164	125.1	31	18	6
27	1600	2223	1735	167	122.6	30	18	6
28	1615	2027	1454	172	133.7	30	19	6
29	1590	2050	2175	154	131.9	29	18	6
30	943	1370	1185	140	82.3	29	16	6
31	567	871	580	139	78.0	27	18	6
32	1491	1734	1416	164	98.5	29	17	6
33	938	1197	1000	148	85.2	30	16	6
34	1790	2263	1816	179	147.8	30	18	6
35	1405	1728	1508	174	146.9	29	20	6
36	1618	2095	1995	178	146.0	32	19	6
37	1	400	80	150	86.0	29	18	10
38	1	80	1	119	92.0	31	19	10
39	1	50	1	117	63.0	30	17	10
40	1	30	1	132	61.5	27	19	1
41	1	38	10	137	96.5	30	19	1
42	1	30	1	120	68.0	29	17	1
43	1	20	5	114	55.0	26	16	1
44	1	49	5	123	69.0	30	17	1
45	1	93	5	130	82.0	30	17	1
46	1	104	11	160	115.5	35	18	1
47	1	35	10	149	107.0	32	18	1
48	390	1090	290	167	135.0	33	21	2
49	340	840	260	153	112.0	31	18	2
50	80	370	35	149	93.0	29	18	2
51	158	530	39	135	74.5	31	21	2
52	175	680	65	148	85.0	31	18	2
53	215	1060	120	161	119.0	32	19	2
54	260	925	160	165	132.0	33	18	2
55	1	40	1	122	52.0	28	15	11
56	1	36	1	117	53.0	27	17	11

OBS	ANALGLND	TESTES	SEMVES	BODYLNTH	BODYWT	HINDFOOT	EAR	MONTH
57	1	35	1	128	59.0	28	17	11
58	1	60	1	145	101.0	30	18	11
59	1	34	1	136	91.3	27	17	11
60	1	63	1	158	121.0	31	18	11
61	60	478	29	138	87.0	32	17	2
62	1	342	10	130	77.0	29	17	2
63	510	1016	703	141	97.0	32	17	3
64	281	765	194	127	63.0	29	17	3
65	1129	960	926	159	95.0	32	19	4
66	1170	1940	1139	162	91.0	33	19	4
67	1080	1660	1220	159	109.0	31	18	4
68	1141	1572	850	140	81.1	28	15	9
69	824	1426	997	134	84.7	30	17	9
70	1615	1700	2310	156	127.7	31	19	9
71	51	365	40	147	112.0	32	18	2
72	333	600	232	172	168.1	32	19	2
73	1	206	1	164	128.6	28	18	2
74	1	206	64	137	72.8	30	19	2
75	320	834	283	157	109.3	30	18	2
76	1	213	20	140	115.5	30	19	2
77	805	1120	620	139	92.2	31	19	3
78	660	950	245	118	78.0	29	18	10
79	2342	1966	2600	158	163.0	31	18	9
80	1	96	10	116	63.0	30	17	9
81	1305	1920	1430	161	122.0	32	18	9
82	530	740	298	160	134.0	31	19	2
83	1	48	1	154	117.0	33	19	12
84	1	41	15	139	83.0	30	18	12
85	1	30	1	145	89.0	30	18	12
86	1	33	1	124	56.0	28	18	12
87	1	45	10	142	95.0	30	18	12
88	1	52	15	145	94.0	31	18	12
89	1	51	15	154	114.0	33	19	12
90	1	38	15	139	89.0	30	18	12
91	1093	1196	1078	152	108.0	31	20	3
92	1770	1540	1595	155	124.0	31	19	4
93	1616	1995	2289	154	111.0	31	19	9
94	421	907	310	121	62.0	31	17	9
95	1234	1177	1313	133	83.0	31	18	9
96	1756	2311	2017	164	126.0	32	20	9
97	1	38	10	130	71.0	28	18	12
98	1	35	5	135	76.0	32	18	12
99	1	40	1	138	81.0	29	18	12
100	1	42	10	132	78.0	28	18	12
101	1	55	10	142	101.0	30	18	12
102	1	40	1	140	112.0	32	19	12
103	1	49	10	141	102.0	32	18	12
104	1	45	10	140	96.0	30	17	12
105	1	38	10	145	104.0	29	17	12
106	1	78	10	146	111.0	32	19	12
107	1	33	5	122	79.0	28	19	12
108	1	42	1	136	96.0	30	18	12
109	1	35	15	130	69.0	28	17	12
110	1	25	10	151	114.0	31	18	12
111	1	54	15	134	87.0	31	18	12
112	1	48	10	140	102.0	32	19	12

OBS	ANALGLND	TESTES	SEMVES	BODYLNTH	BODYWT	HINDFOOT	EAR	MONTH
113	1	35	5	138	97.0	30	19	12
114	1000	1200	120	150	91.5	28	15	10
115	1340	1400	1350	149	89.0	32	17	8
116	1190	1060	1580	157	102.0	31	18	8
117	1180	1010	1280	149	86.0	30	16	8
118	760	840	780	147	89.0	31	18	7
119	790	920	500	140	79.0	31	17	7
120	1	450	163	125	55.0	29	16	7
121	1	580	210	137	75.0	29	17	7
122	20	310	50	125	56.0	30	14	7
123	940	880	1220	135	88.0	31	16	7
124	370	760	280	136	68.0	29	16	7
125	230	500	90	142	74.0	31	17	7
126	1060	1060	720	144	88.0	30	17	7
127	1410	1240	1430	149	88.0	30	17	7
128	1603	1525	1955	185	119.0	32	17	6
129	770	880	670	146	73.0	30	17	6

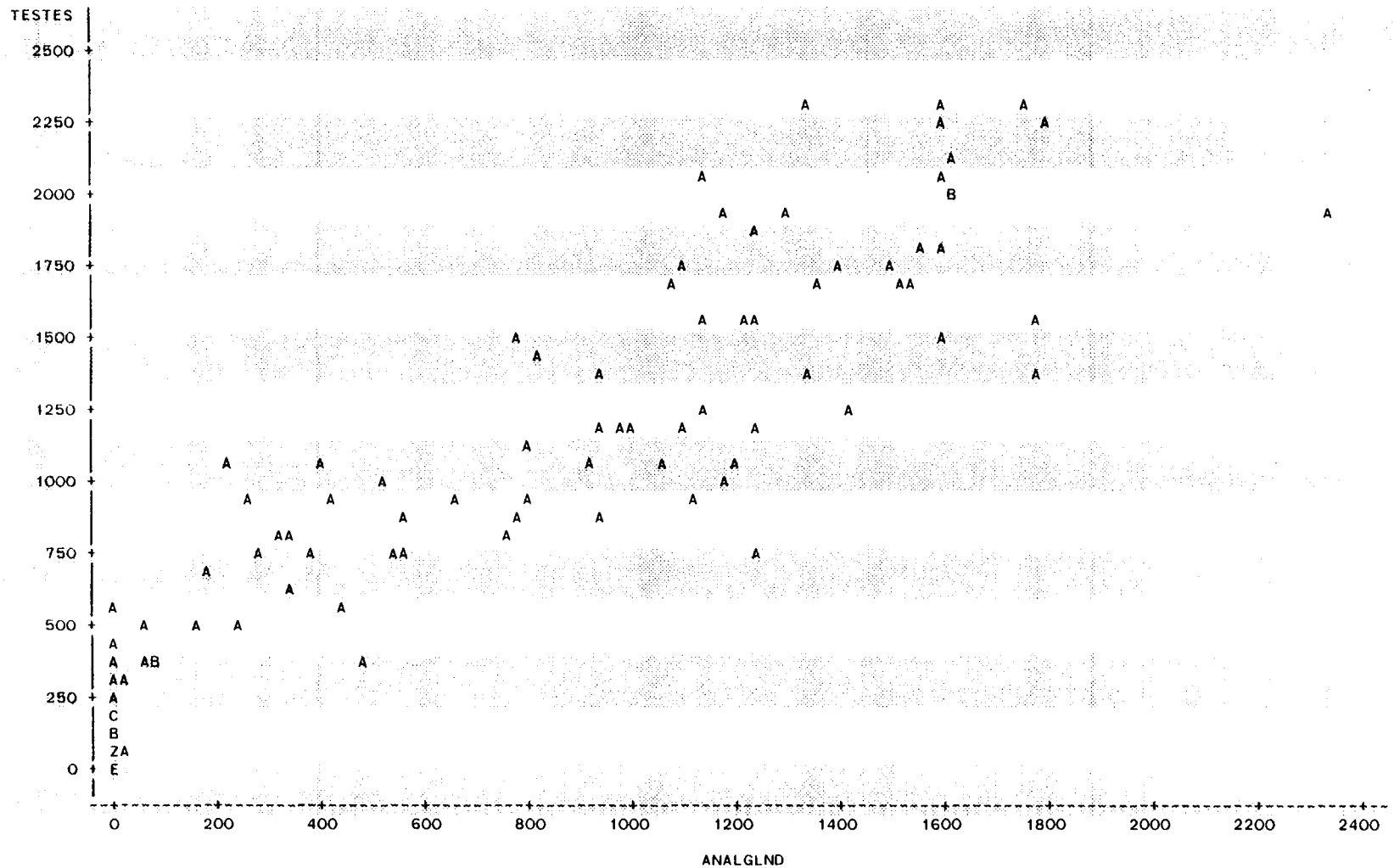
Appendix B - Plots of Testes and Seminal Vesicles Weights versus Perineal Gland Weights

Plot of TESTES*ANALGLND. Symbol used is '*'.
Plot of SEMVES*ANALGLND. Symbol used is 'o'.



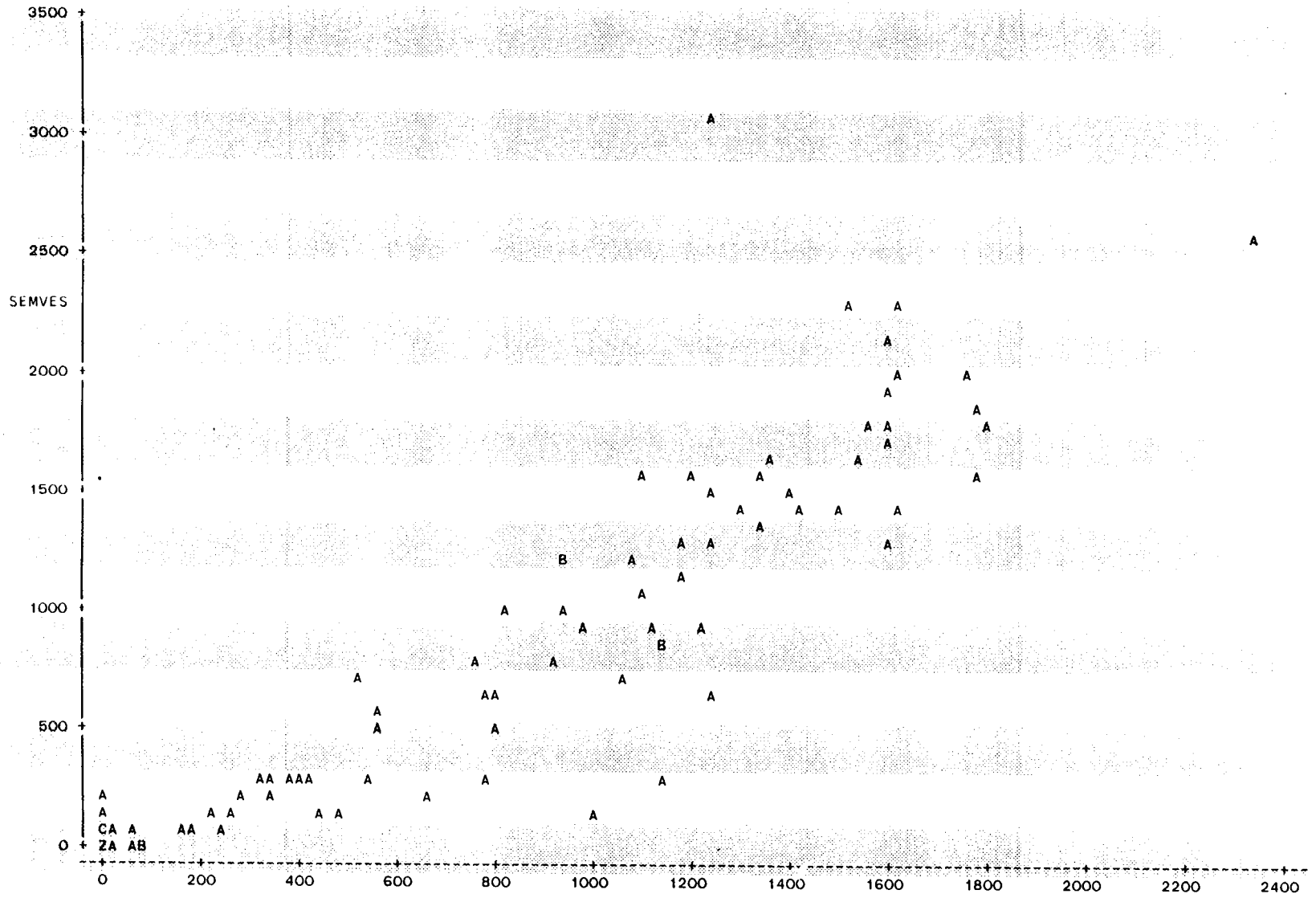
NOTE: 103 obs hidden.

Plot of TESTES*ANALGLND. Legend: A = 1 obs, B = 2 obs, etc.



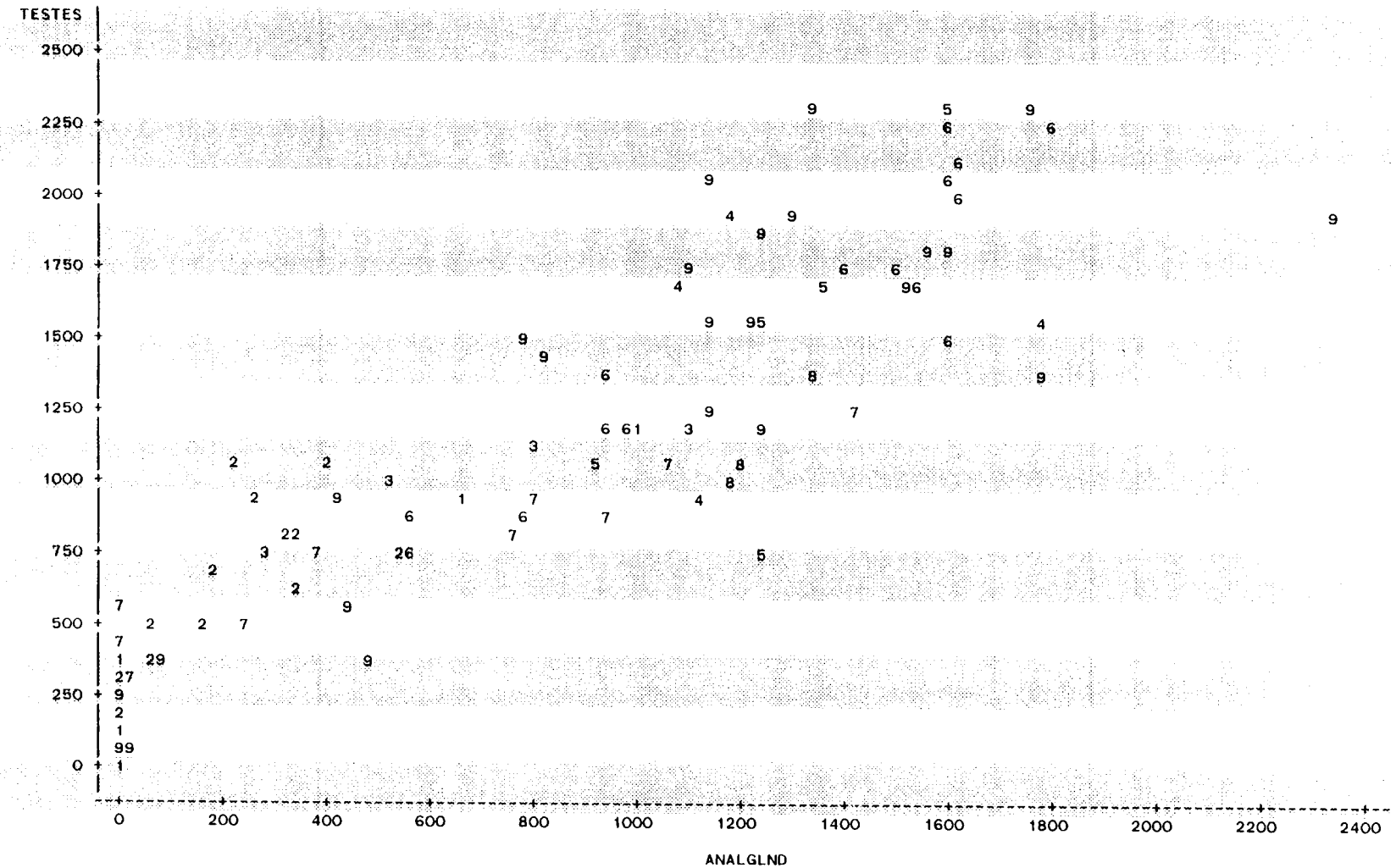
NOTE: 12 obs hidden.

Plot of SEMVES*ANALGLND. Legend: A = 1 obs, B = 2 obs, etc.



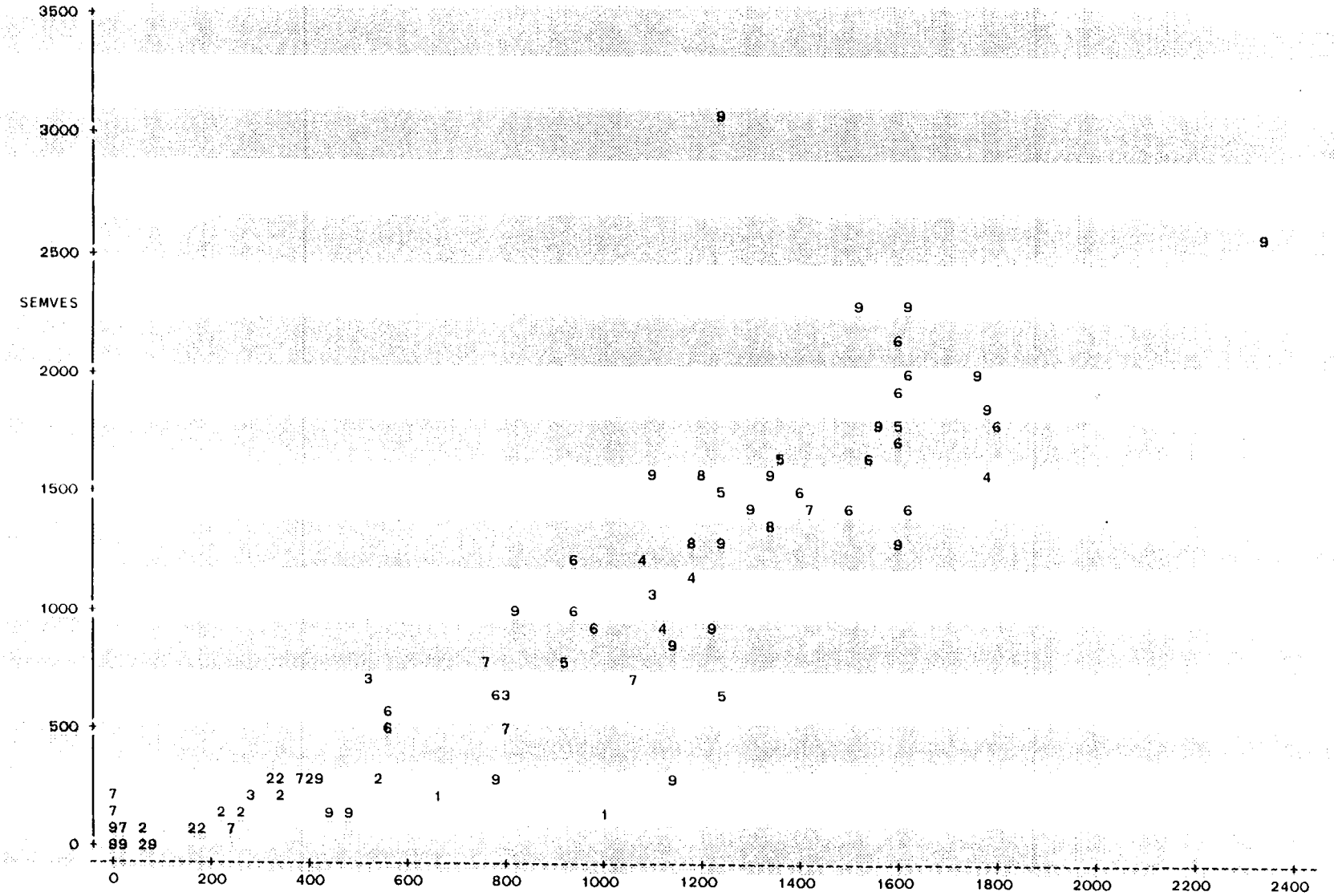
NOTE: 22 obs hidden.

Plot of TESTES*ANALGLND. Symbol is value of MONTH.



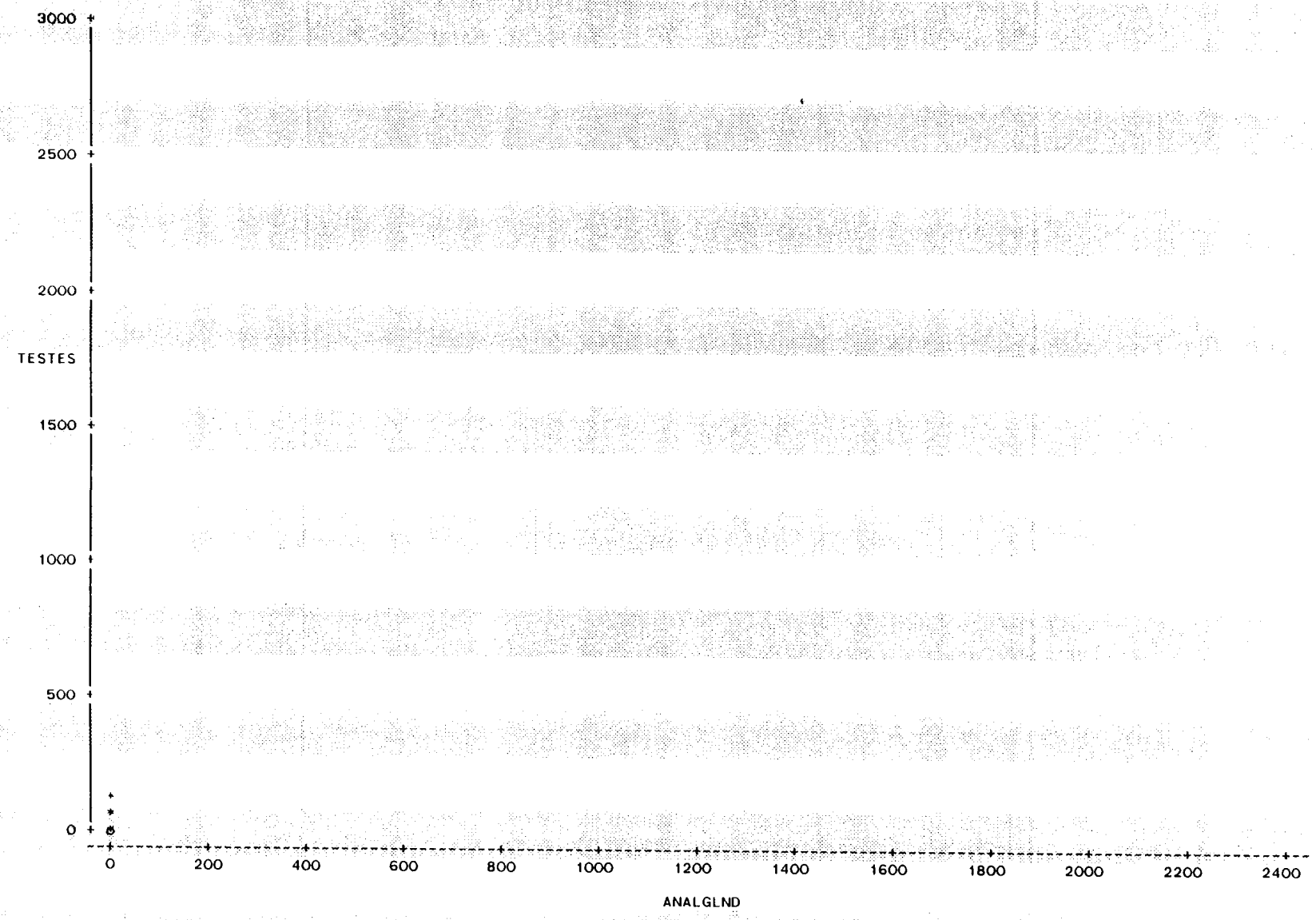
NOTE: 46 obs hidden.

Plot of SEMVES*ANALGLND. Symbol is value of MONTH.



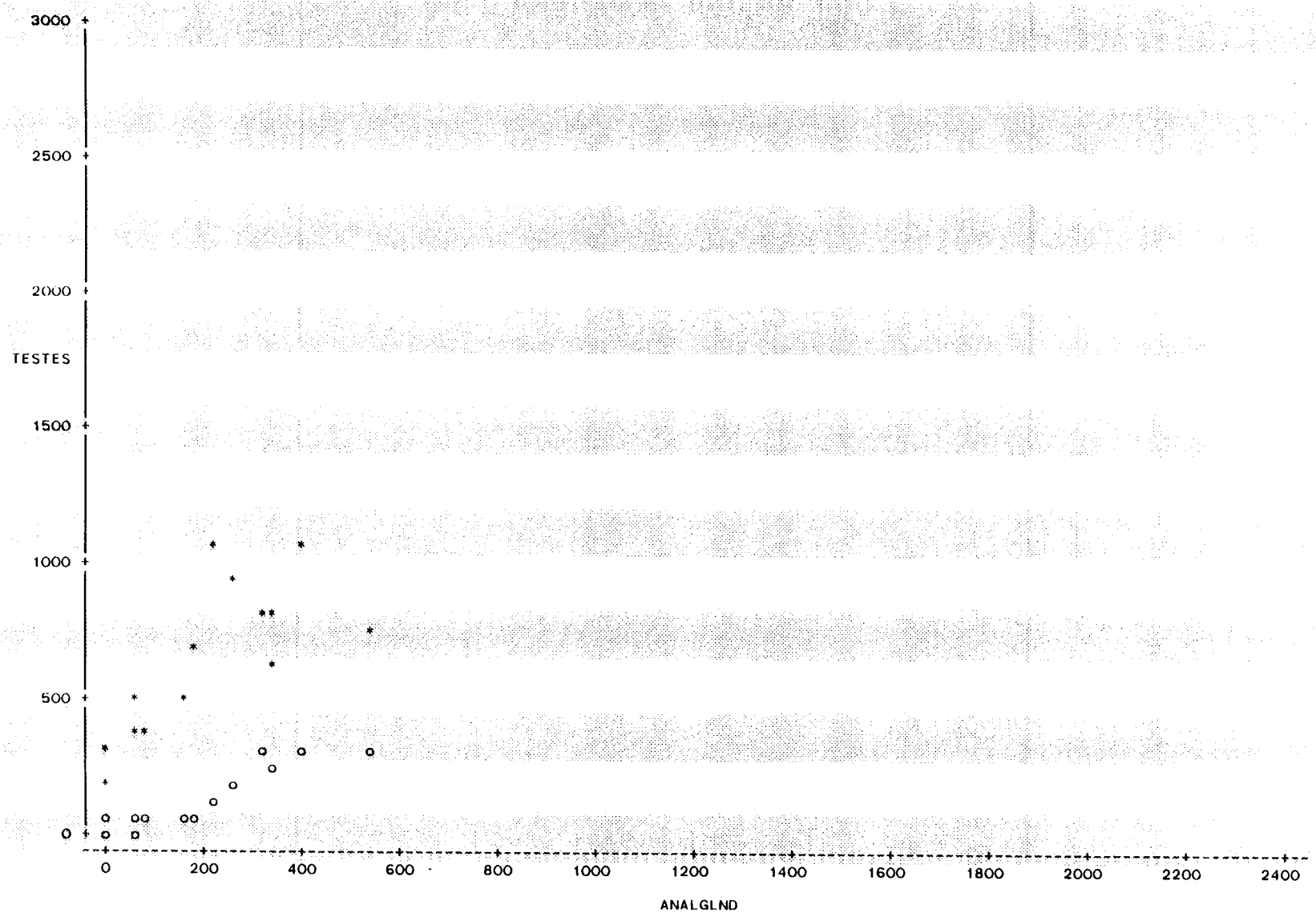
NOTE: 52 obs hidden.

Plot of TESTES*ANALGLND. Symbol used is '*'.
Plot of SEMVES*ANALGLND. Symbol used is 'o'.



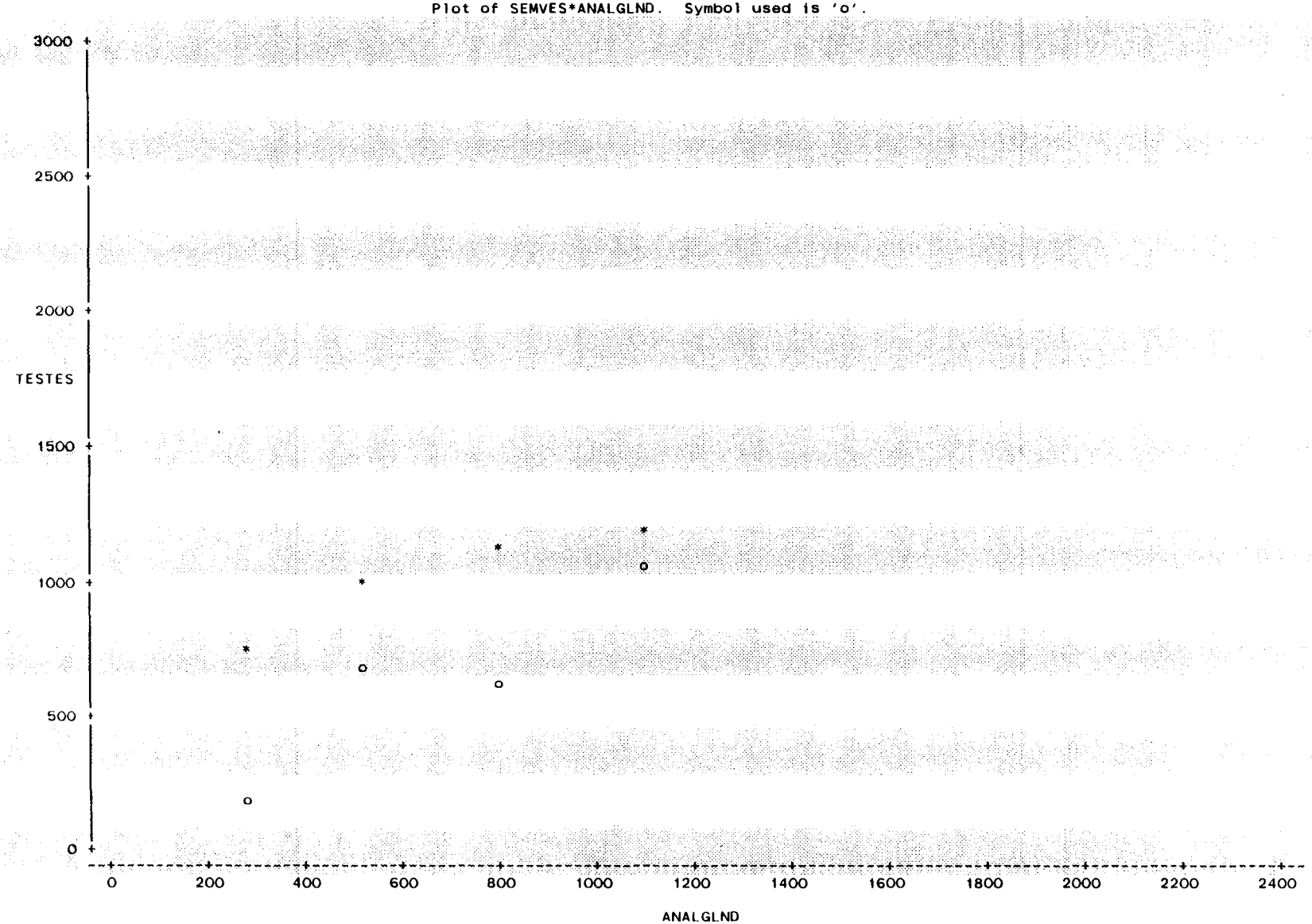
NOTE: 12 obs hidden.

Plot of TESTES*ANALGLND. Symbol used is '*'.
Plot of SEMVES*ANALGLND. Symbol used is 'o'.

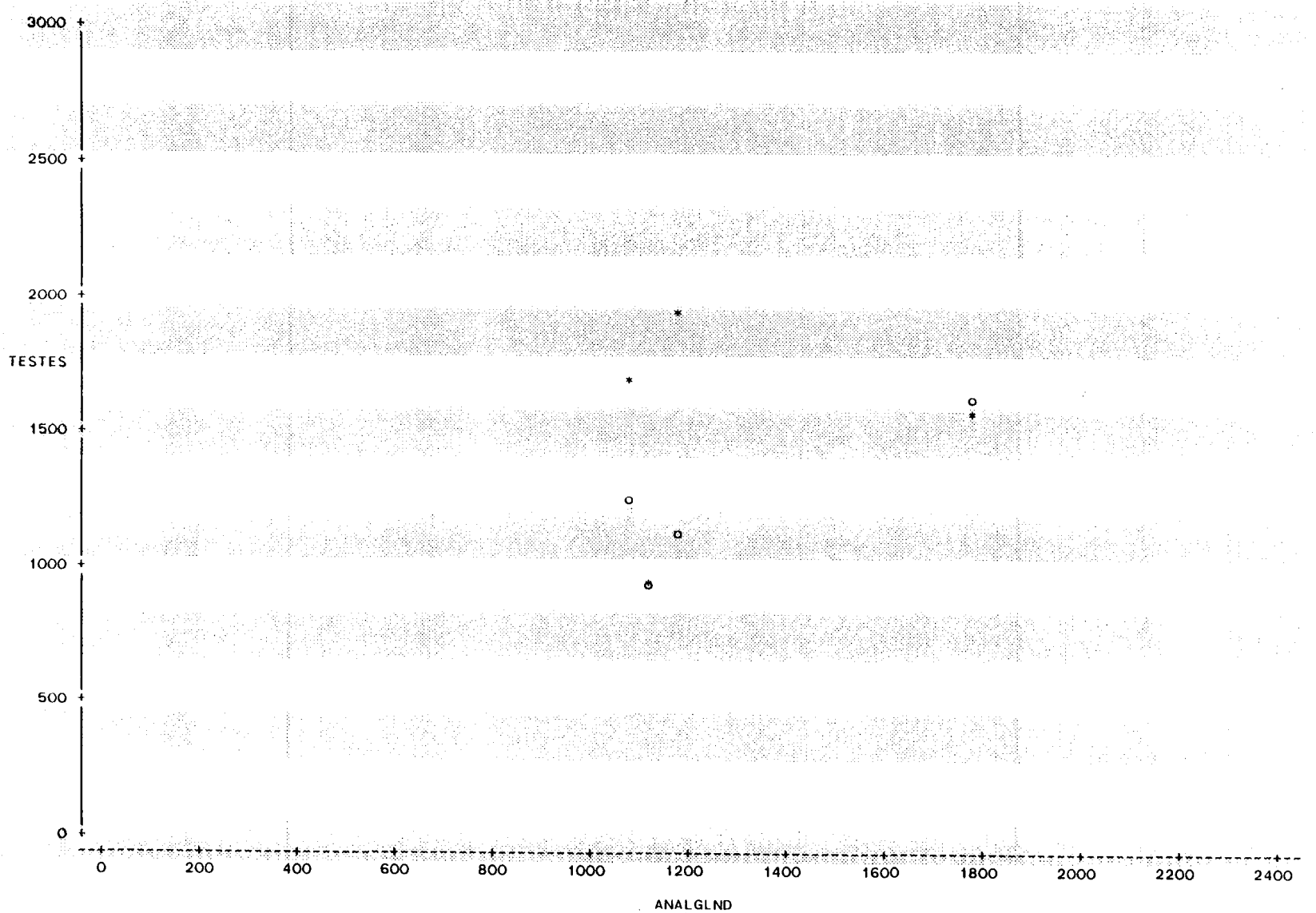


NOTE: 5 obs hidden.

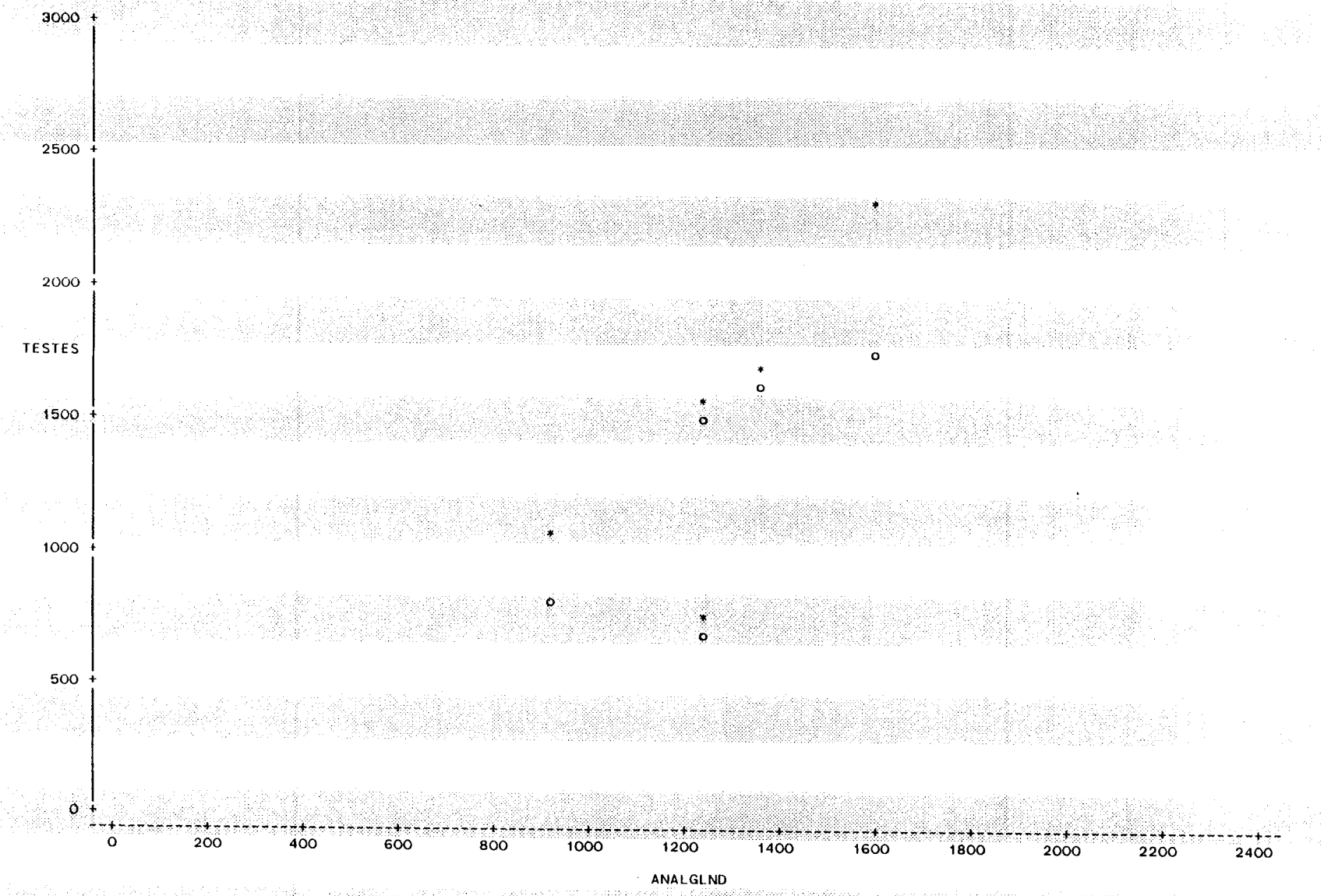
Plot of TESTES*ANALGLND. Symbol used is '*'.
Plot of SEMVES*ANALGLND. Symbol used is 'o'.



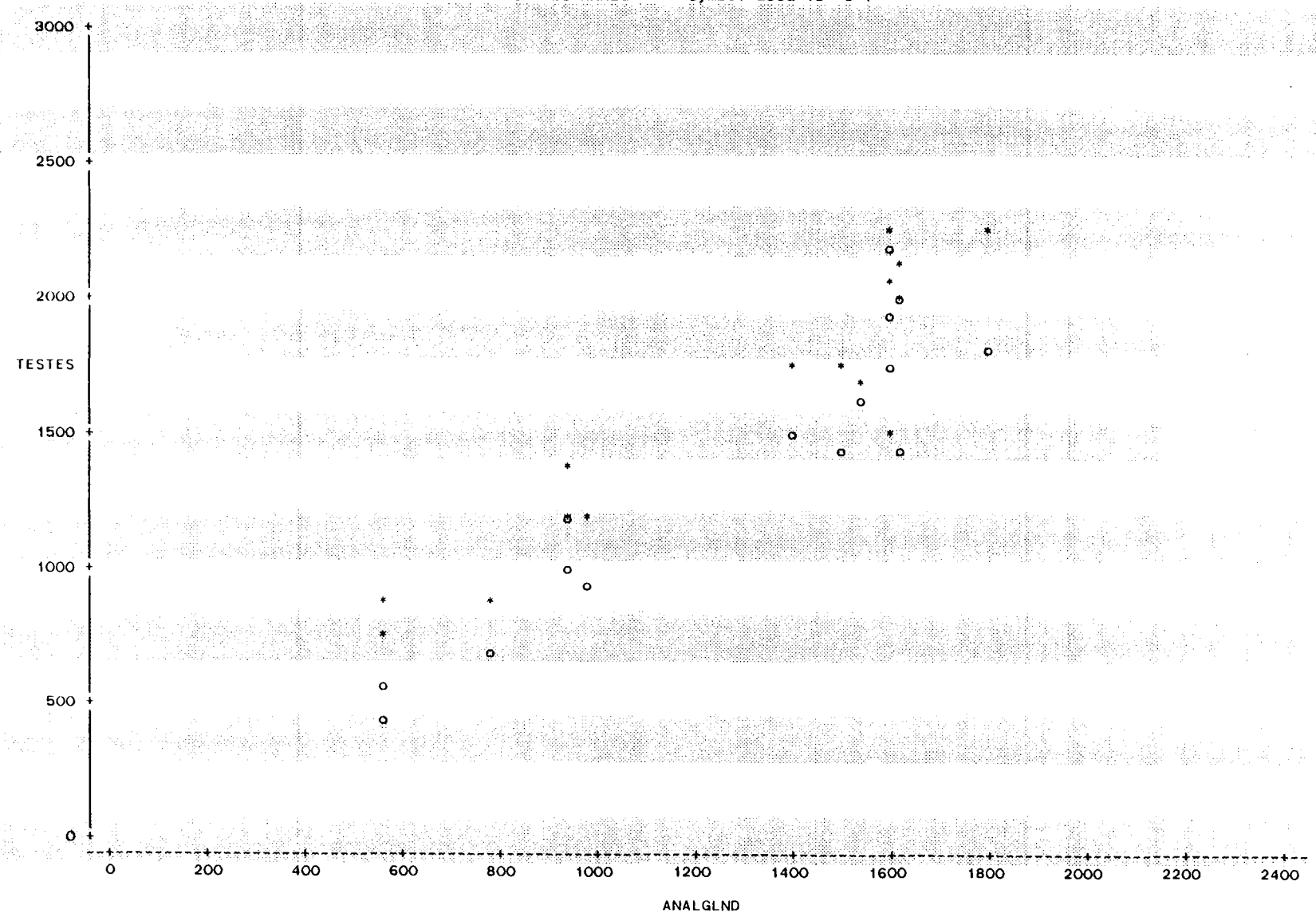
Plot of TESTES*ANALGLND. Symbol used is '*'.
Plot of SEMVES*ANALGLND. Symbol used is 'o'.



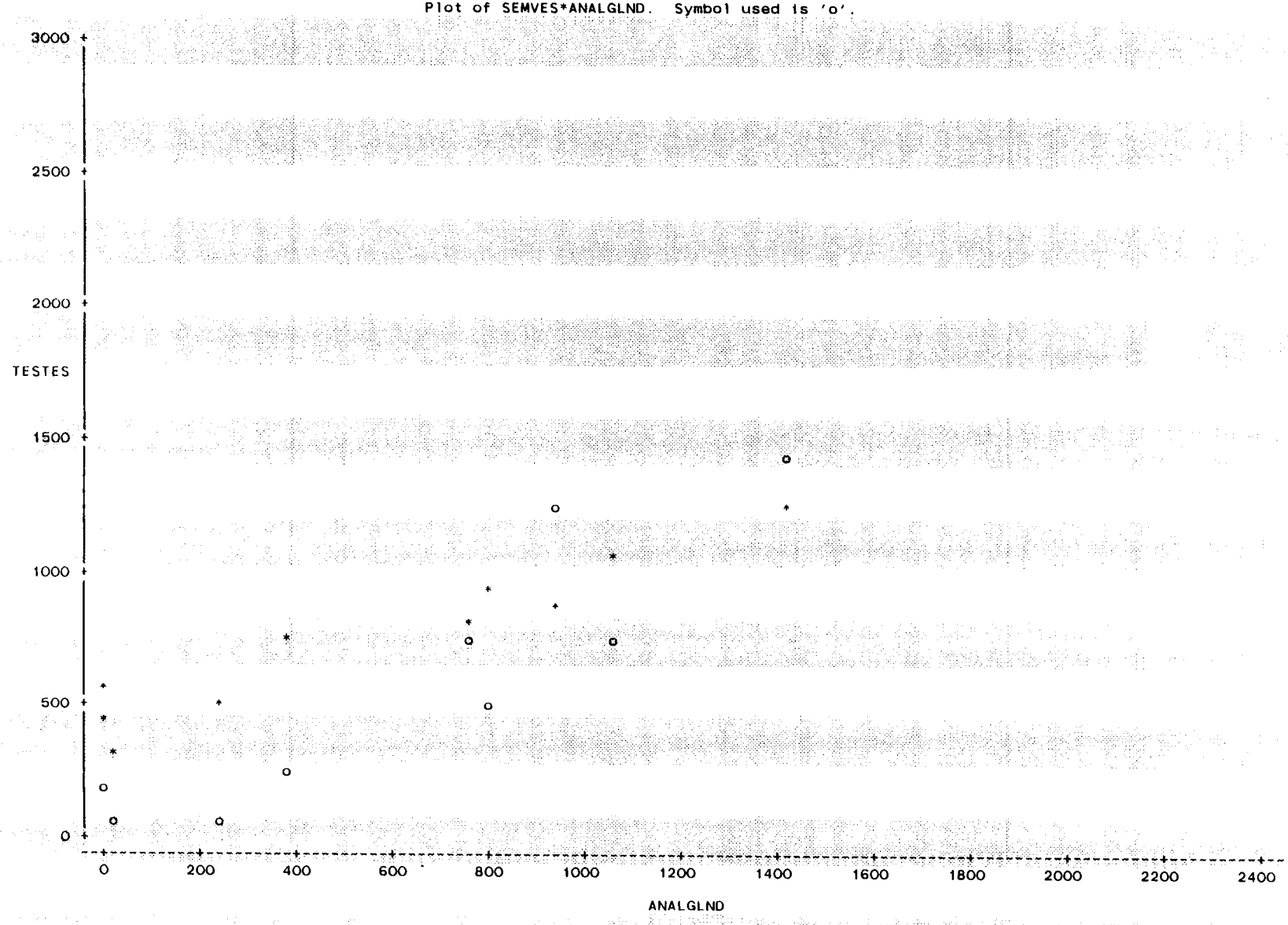
Plot of TESTES*ANALGLND. Symbol used is '*'.
Plot of SEMVES*ANALGLND. Symbol used is 'o'.



Plot of TESTES*ANALGLND. Symbol used is '*'.
Plot of SEMVES*ANALGLND. Symbol used is 'o'.

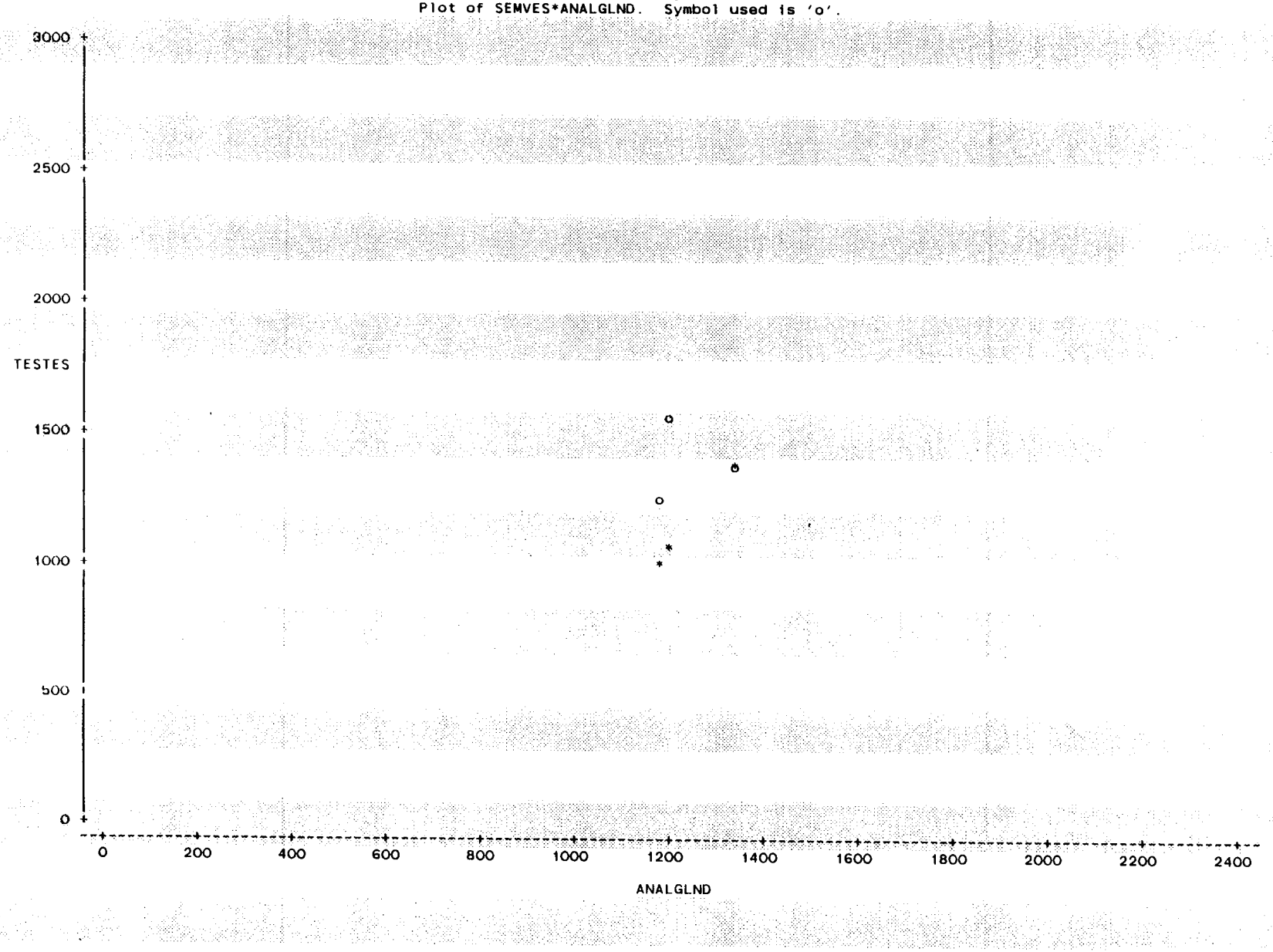


Plot of TESTES*ANALGLND. Symbol used is '*'.
Plot of SEMVES*ANALGLND. Symbol used is 'o'.

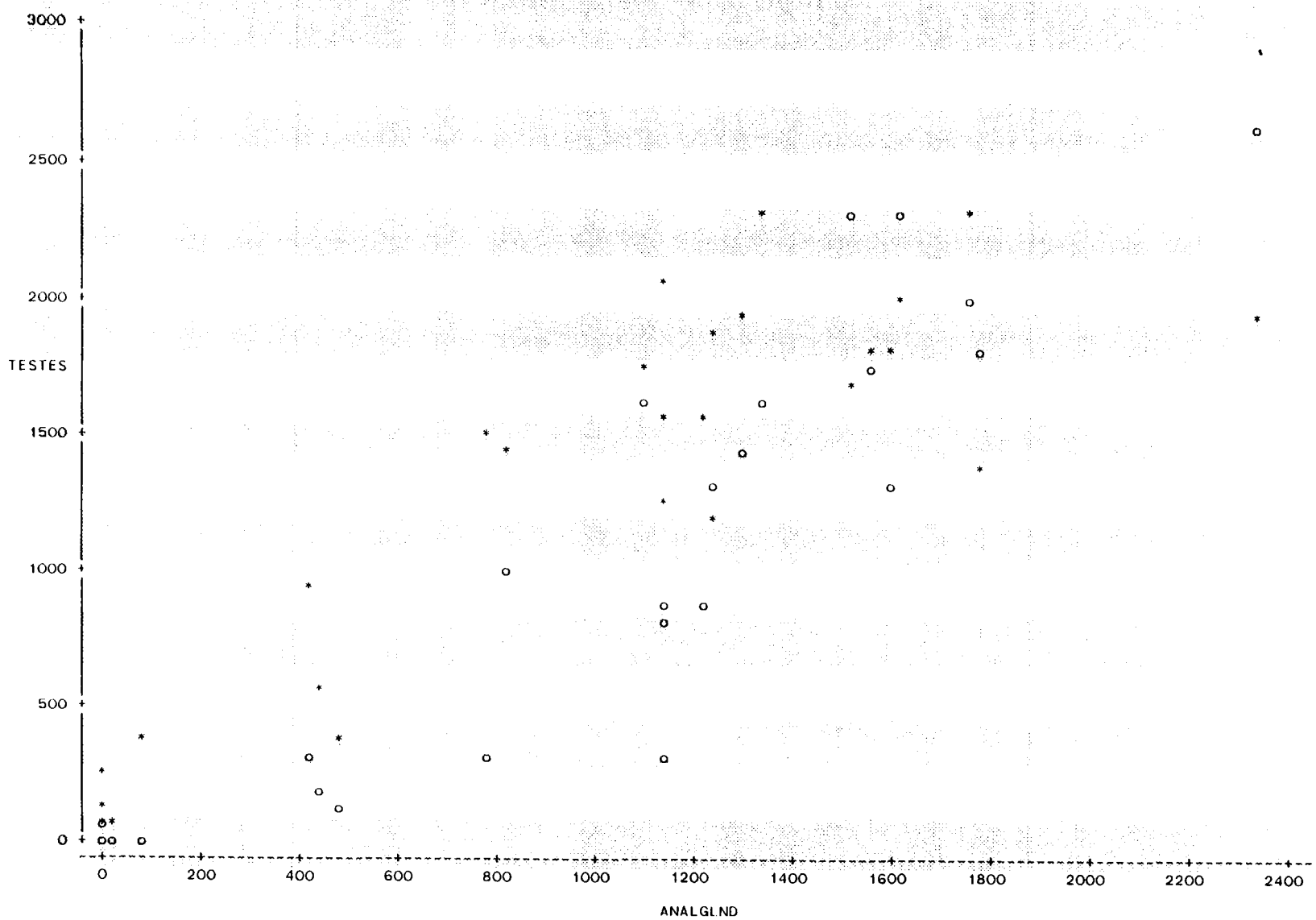


NOTE: 1 obs hidden.

Plot of TESTES*ANALGLND. Symbol used is '*'.
Plot of SEMVES*ANALGLND. Symbol used is 'o'.

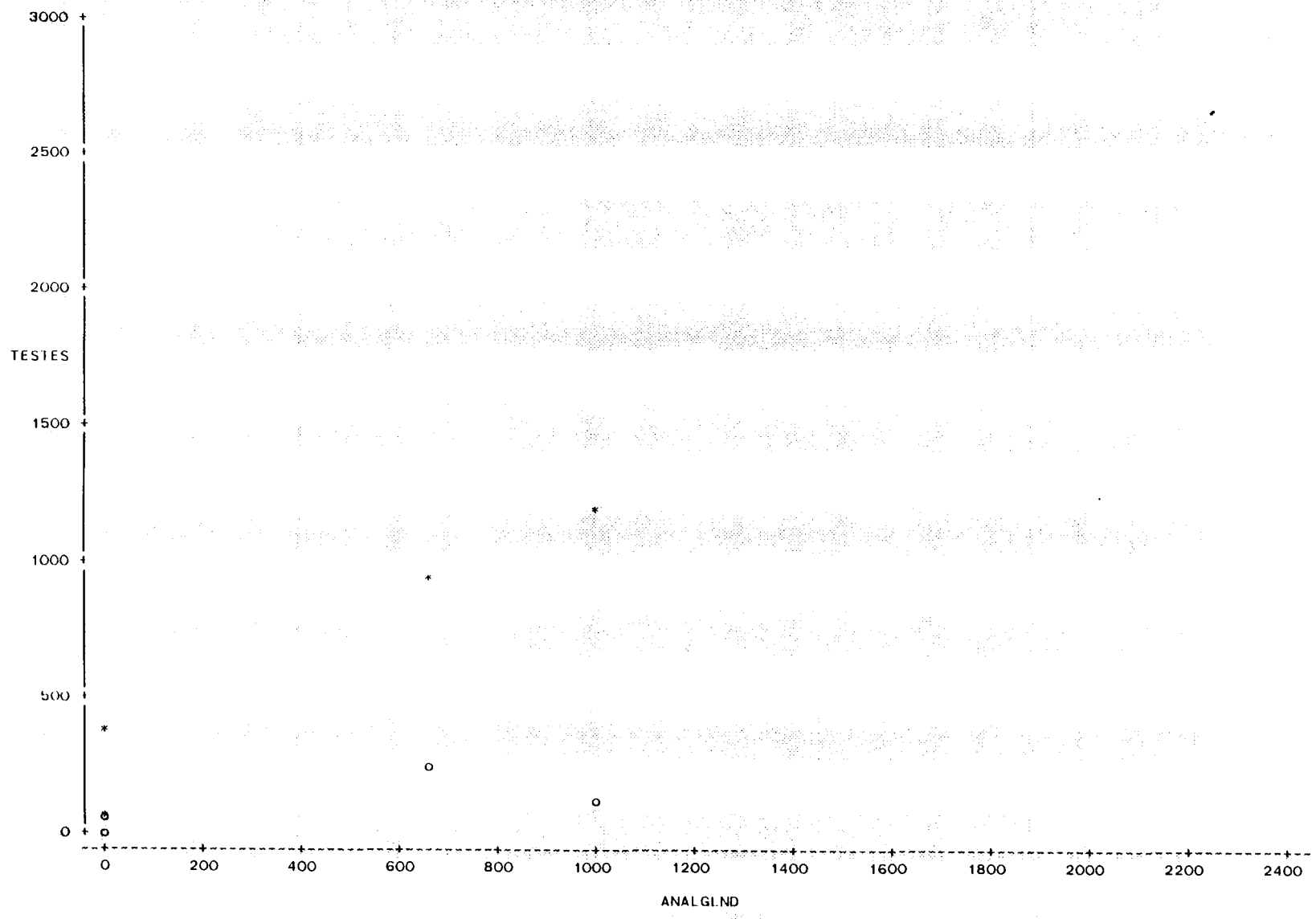


Plot of TESTES*ANALGLND. Symbol used is '*'.
Plot of SEMVES*ANALGLND. Symbol used is 'o'.



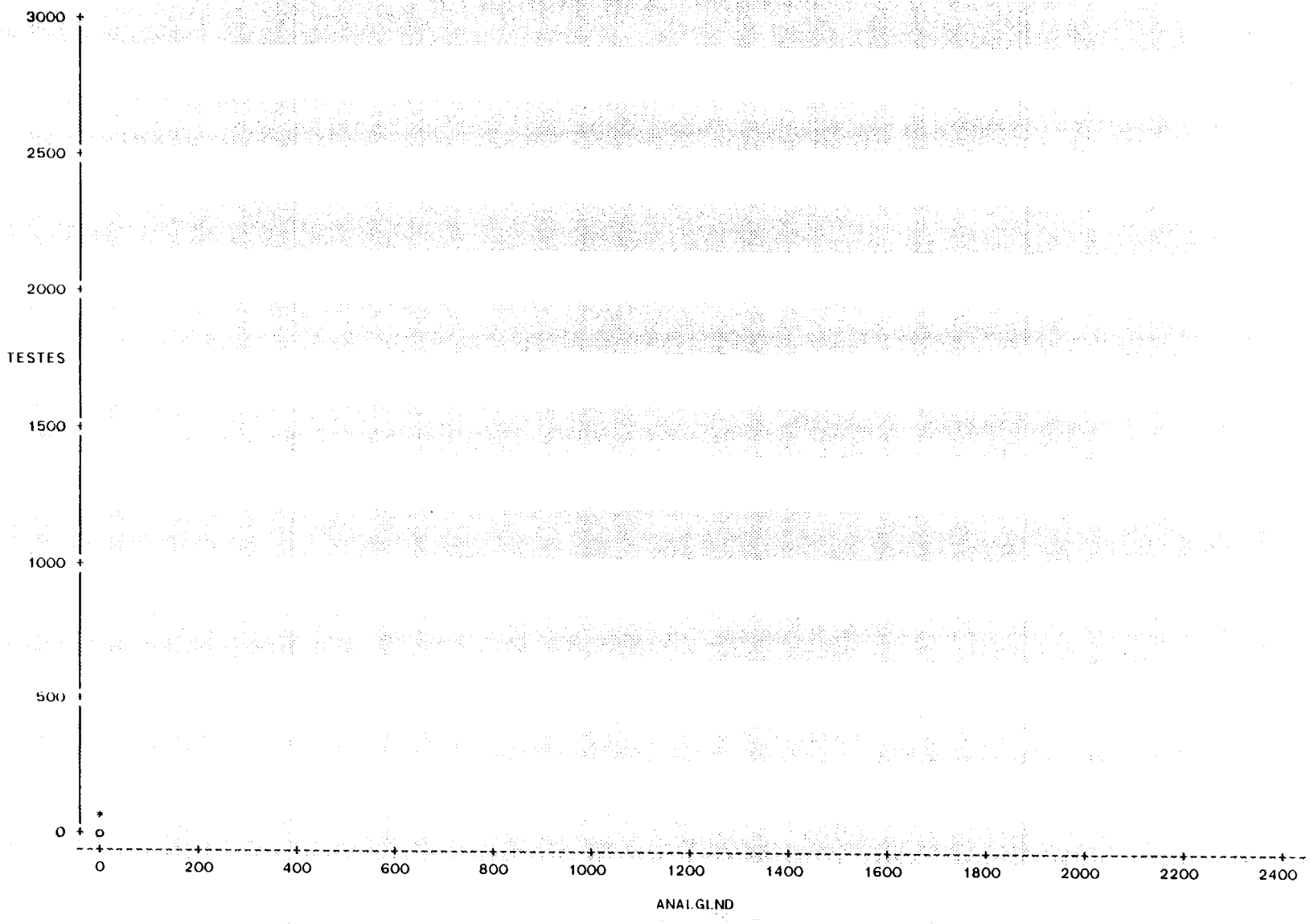
NOTE: 5 obs hidden. 1 obs out of range.

Plot of TESTES*ANALGLND. Symbol used is '*'.
Plot of SEMVES*ANALGLND. Symbol used is 'o'.



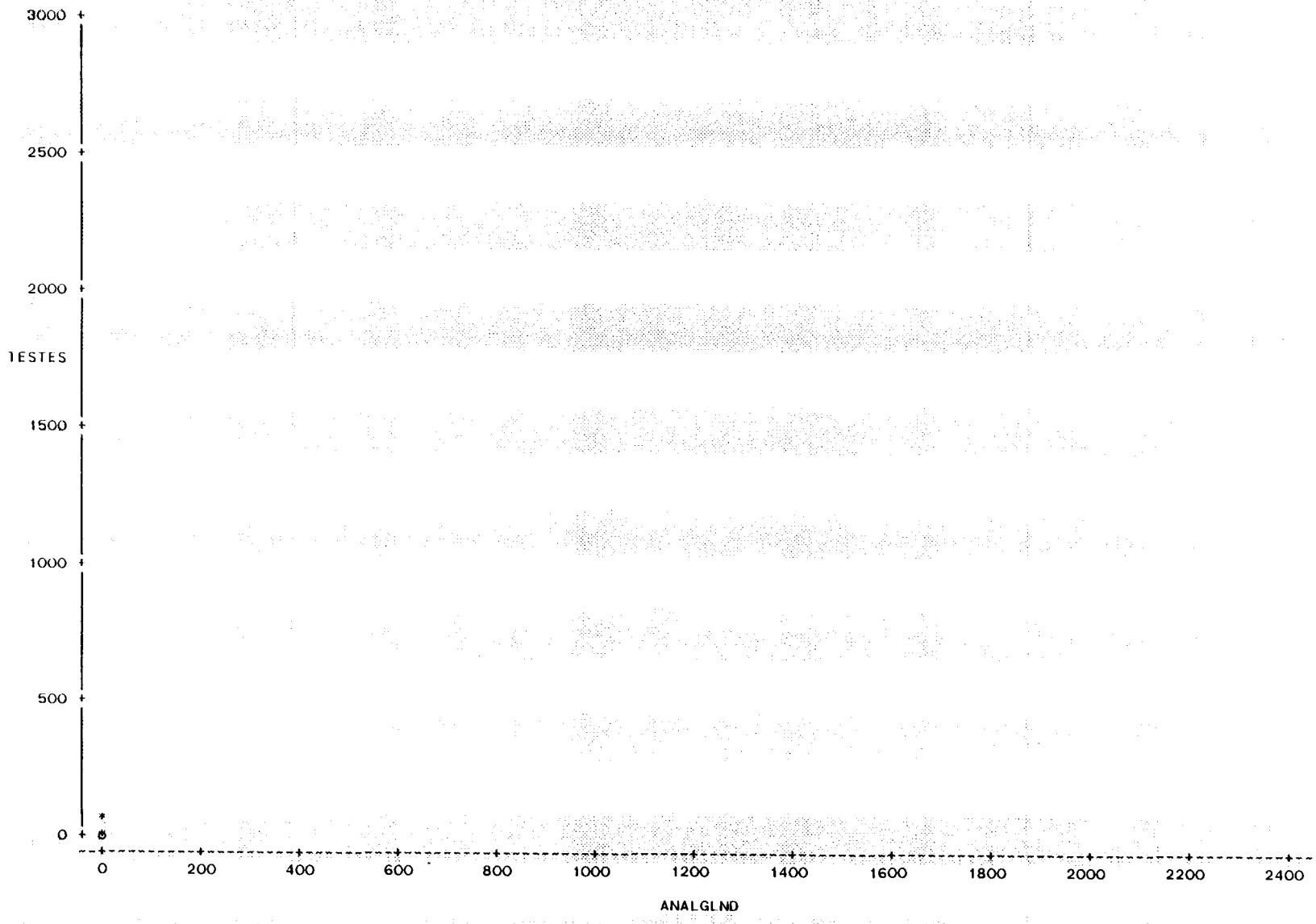
NOTE: 2 obs hidden.

Plot of TESTES*ANALGLND. Symbol used is '*'.
Plot of SEMVES*ANALGLND. Symbol used is 'o'.



NOTE: 10 obs hidden.

Plot of TESTES*ANALGLND. Symbol used is '*'.
Plot of SEMVES*ANALGLND. Symbol used is 'o'.



NOTE: 47 obs hidden.