SEASONAL CHANGES IN SOME ENDOCRINE ORGANS OF THE VOLE (*MICROTUS AGRESTIS*)

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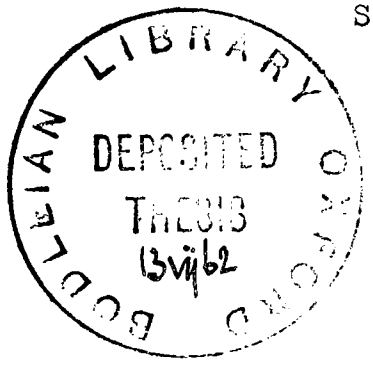

ABSTRACT

Seasonal Changes in some Endocrine Organs of the Vole (Microtus agrestis)

Considerable progress has been made in determining the factors in the environment which are responsible for the control of seasonal breeding in mammals and birds. There is less information available about how these factors produce their physiological effects. Light has been shown to be very important in the control of vole breeding seasons. Temperature may also have some effect. How these factors act is not known. As part of an attempt to understand how the breeding season of the vole is controlled, an investigation of some of its endocrine organs has been undertaken.

Animals have been collected from the field in mid-summer (at the height of the breeding season) and in mid-winter (during the non-breeding period). A total of 174 adult field animals has been studied. As an approximate indication of the reproductive and endocrinological state of the animals, measurements have been made of the weights of their bodies, adrenals, thyroids, gonads and also certain accessory organs (the uteri of females in winter and the seminal vesicles and ventral prostates of males in both summer and winter). From the examination of this data it is clear that there are considerable endocrine changes with season. Furthermore, within both winter and summer

populations reproductive sub-groups which show endocrine differences can be recognized. More detailed study of these endocrine differences has involved the use of cytological and histological methods.

Because of the trophic control which the adenohypophysis exercises over the other endocrine organs, it may be presumed to be important in bringing about seasonal changes. It has, therefore, received particular study. The adenohypophyses of field animals have been examined by the methods of cytology, histochemistry and bioassay. In order to identify the cells responsible for the production of gonadotrophin and thyrotrophin, the pituitary function of laboratory bred animals has been altered by castration and the administration of goitrogens.

In the adenohypophysis of the vole five cell types have been recognized.

1. Oval basophils which are periodic acid-Schiff (PAS) positive and aldehyde fuchsin (AF) negative. They react to castration by degranulation and the subsequent formation of colloid filled castration cells. They are considered to secrete gonadotrophins. In field animals it has been found that the gonadotrophin producing cells (gonadotrophs) of winter animals are markedly different from those of summer animals. The gonadotrophin content of the pituitaries of male voles in winter and in summer has been studied by means of bioassay. In the summer the vole

pituitary contains well granulated gonadotrophs and gonadotrophic hormone can be detected. In winter the gonadotrophs are vesiculated and no gonadotrophic hormone can be detected. This indicates that the granulated gonadotrophs contain hormone, but that the vesiculated cells are depleted of active gonadotrophic principles.

2. Angular basophils which are PAS positive and AF positive. They react to the administration of goitrogen by the formation of colloid filled thyroidectomy cells. They are considered to secrete thyrotrophin. They show no marked changes with season, as would be anticipated from the lack of marked change in the thyroid itself.

3. Round acidophils which show no marked change with season. This study provides no indication as to their function.

4. A second type of acidophil which is numerous and well developed only in females which are pregnant or show signs of mammary development. It is suggested that they may be the source of prolactin.

5. The adenohypophysis also contains a few large cells with poor staining qualities. They do not show any marked changes with season or in response to either castration or the administration of goitrogens. Their function is not known.

The gonads were, in general, found to exhibit the expected seasonal change in activity. Sexually inactive males in winter

may be divided into two groups on the basis of the structure of the tunica albuginea. It is suggested that these two groups represent, respectively, regressed males, which were sexually active in the preceding summer, and inhibited males, which were born late in the season and have never been sexually active. Similarly, on the basis of uterine weight, female voles in winter can be divided into two groups, parous and non-parous. The pituitary cytology of regressed and inhibited males, parous and non-parous females in winter is similar.

In one winter collection the males show considerable evidence of being sexually active. This suggests the operation of a factor or factors other than light and temperature in the control of vole breeding seasons. These males were distinguished from the males in other winters by differences in pituitary cytology.

The study of the pituitary suggests that gonadal changes at the end of the breeding season are secondary to changes in the pituitary. The alternative possibility, that the gonads are not competent to respond to pituitary hormones, was tested experimentally. Commercial gonadotrophins were injected into winter field animals and into laboratory bred animals whose sexual development had been inhibited by maintaining them on short days in the cold. The results suggest that the gonads of such animals are able to respond to gonadotrophins.

The adrenal has been found to undergo marked changes in weight with season. In summer there is also a sex difference in adrenal weight. These weight changes can be correlated with striking histological differences in the inner regions of the adrenal cortex. In winter voles the adrenal cortex possesses a juxtamedullary zone. It is similar in cytological appearance to the X zone of mice and the two zones are seemingly homologous. The zone is present in all winter field voles, whether regressed or inhibited males, parous or non-parous females. The zone is small or absent in the adrenals of sexually active males. It must, therefore, be formed secondarily in the adrenals of regressed males. It also reappears in a similar, though not identical, form in males after castration. The zone can also disappear from females, but is present in an especially well developed form in all pregnant and lactating animals. Multipara and primipara differ in the details of the structure of the juxtamedullary zone. These changes are clearly related to sexual activity, but their significance is not known.

The epithelium lining the ventral prostate is the site of marked stimulation in winter field males and in castrated laboratory animals. It is possible that the adrenal is the source of the hormone responsible for this stimulation.

The zona glomerulosa and zona fasciculata of the adrenal cortex also show cytological and histological change with

season. Their structure in the winter males which may have been sexually active suggests that these changes are not primarily related to sexual activity.

The principal conclusions which can be drawn from this study are:

1. That in the vole there is functional differentiation of adenohypophyseal cells. Two cell types have been recognized which are clearly related to the production and secretion of gonadotrophic and thyrotrophic hormones, respectively. A third cell type is probably the source of prolactin. There is no evidence available on the function of the other two cell types which have been recognized.

2. That the cessation of breeding in winter in the vole is apparently brought about by a cessation of both the synthesis and the secretion of gonadotrophin.

3. That there are striking changes in the juxtamedullary region of the vole adrenal which appear to be related to sexual activity.

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ACKNOWLEDGEMENTS

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INTRODUCTION

Very many species of mammals and birds, from all parts of the world, breed only during part of the year. This is apparently of adaptive significance. Where detailed investigations have been made, it has been found that the time of mating is such that young are born or eggs hatch at the most favourable time of year, when, for example, food supply is most abundant. Young born outside the breeding season will have less chance of survival than young born within it. Indeed, it may not be possible for them to survive at all (see Lack, 1954).

Between fertilisation and the appearance of young there intervenes a period necessary for embryonic development. In birds, monotremes and marsupials this is relatively short, but in Eutherian mammals it may be many months. Thus mating may take place at quite a different season from birth. Such considerations lead Baker (1938) to distinguish between the ultimate and proximate factors involved in the control of breeding seasons. The ultimate factors are those, such as availability of an adequate food supply for the young and of suitable breeding sites or nesting materials, which are concerned with the survival value of breeding seasons. Proximate factors are those which determine when the gonads shall become active and mating and breeding activity be initiated.

Ultimate and proximate factors may be the same. This seems to be the case in xerophilous birds which are opportunist breeders. The environmental conditions, which follow rainfall and which enable young to be reared successfully, may also be the stimulus to gonad development in such species (Marshall and Serventy, 1958). However, proximate factors are often quite different from ultimate ones.

In temperate climates light is clearly of great importance as a proximate factor in the control of breeding seasons. This was first shown by the experiments of Rowan (1926) with the junco, Junco hyemalis. The problem of the cessation of breeding seasons has been much less studied, but the recent experiments of Wolfson (1959) have indicated how light could control the entire reproductive cycle of both migratory and non-migratory birds.

The vole, Microtus agrestis, is a seasonally breeding animal. This was shown by the field studies of Baker and Ranson (1933) and of Brambell and Hall (1939), which indicated that in Britain the breeding season of the vole lasts from February or March to September or October.

Baker and Ranson (1932a, 1932b) showed in laboratory experiments that light, with temperature a possible modifying factor, is most important in the control of the breeding season in the vole. However, it is possible that light and temperature are not the only factors involved. Social inter-

action may be important in determining when animals breed. Chitty (1952) studied the cyclic changes in population size which field voles undergo. He presented evidence that the size of a population can influence the length of the breeding season and the fecundity of animals, as well as their longevity. In the summer preceding a marked decline in numbers, the breeding season ended in August, a month earlier than expected. In the spring of the following year, when animals were very scarce, there were areas in which no females bred. Clarke (1955) had evidence of similar effects in two experimental vole populations of different size.

Considerable progress has thus been made in identifying the proximate factors which are responsible for the control of breeding seasons in the vole, in other species of mammal, for example the ferret (Bissonnette, 1936; Hill and Parkes, 1933b; Hart, 1951) and the sheep (Yeates, 1949; Hart, 1950; Hafez, 1952; Dutt and Bush, 1955; Dutt and Simpson, 1957), and in birds.

In no animal is there anything approaching complete information available about how the proximate factors produce their physiological effects. However, general endocrinological studies have provided concepts which clearly help in understanding the causation of breeding seasons. Thus, it has been firmly established that the adenohypophysis produces trophic hormones which influence the gonads (see Evans and Simpson, 1950).

It is, therefore, probable that the adenohypophysis is involved in the mechanism whereby the gonadal changes, which occur in relation to the breeding season, are brought about.

Hill and Parkes (1933a) showed that, if male and female ferrets were hypophysectomized during the non-breeding period, none of the normal spring breeding changes occurred. They also showed (1933b) that hypophysectomy prevented the oestrus response of sexually inactive female ferrets to additional illumination in winter.

If the adenohypophysis is important in bringing about seasonal change in the gonads, then it must show seasonal change in its gonadotrophic function. The adenohypophysis also exercises trophic control over the thyroid and adrenal. If thyroid or adrenal function show change in relation either to the breeding season, or directly to annual fluctuation in any aspect of the external environment, then the adenohypophysis may again show changing function, It is evident that study of the pituitary is of central importance in any study of seasonal change in endocrine organs.

One of the possible methods available for the study of the pituitary is the study of its cytology. Schönemann (1892) classified adenohypophyseal cells into acidophils, which stained with acid dyes, cyanophils or basophils, which stained with basic dyes, and chromophobes, which did not stain. The subsequent work of various authors showed that species differ

both in the relative proportions in which the different cell types occur, and in the way in which they are distributed in the adenohypophysis. Within a species differences in the relative proportion of cells also occur in response to different physiological states, such as castration and pregnancy (see Severinghaus, 1939 for references). Using trichrome methods, for example the method of Crossmon (1937), it is possible to distinguish more than three cell types in the adenohypophysis. As many as five or six cell types have now been distinguished in the adenohypophyses of a number of mammalian species, for example: man (Romeis, 1940), the rat (Purves and Griesbach, 1951a, 1952), the dog (Wolfe, Cleveland and Campbell, 1932; Goldberg and Chaikoff, 1952; Purves and Griesbach, 1957a), the bat (Herlant, 1956), the ferret (Holmes, 1959, 1960), the sheep (Clarke and Purves, 1960; Clarke, 1961).

Pearse (1952) has outlined the methods which have been used in the attempt to correlate the secretory function of the adenohypophysis with its cytology. These have included studies of the cytology of the pituitary in pathological, physiological and various artificially induced states, of the effects of implantation of pituitary material and administration of extracts of the pituitary. Use has also been made of the McManus-Hotchkiss periodic acid-Schiff technique (McManus, 1946). This is a cytochemical method. If the periodic acid-Schiff (PAS) method is applied to the adenohypophysis,

the granules of certain cells react strongly and are magenta in colour. The PAS reaction reveals a number of types of substances, but in this context it is considered that PAS is reacting with the glycoprotein hormones, thyrotrophin, follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Pearse, 1952). Therefore, the adenohypophyseal cells which contain granules reacting strongly with PAS are inferred to be the cells responsible for the production of these hormones. It has been shown that purified preparations of FSH and LH react in vitro with PAS to give a magenta colour (Mellors and Hotchkiss).

Purves and Griesbach (1951b, 1954) and Halmi (1952) have applied the PAS method to rat pituitaries. They agree in distinguishing two kinds of cell which show a positive reaction with PAS, one angular, the other round or oval. The angular cells stain specifically with the aldehyde fuchsin (AF) of Gomori (1950), as well as reacting with PAS. The basis of this staining reaction is not known. The angular PAS and AF positive cells undergo changes in relation to variations in the level of thyroxine secretion. They are considered to be the source of thyrotrophin. They have been termed thyrotrophs by Purves and Griesbach (1951a), delta cells by Halmi (1952).

The round or oval cells react to castration and the administration of oestrogens. They are considered to be the source of gonadotrophins and have been termed gonadotrophs by Purves

and Griesbach (1951a), beta cells by Halmi (1952).

Purves and Griesbach (1952, 1955) further divide gonadotrophs into two types: peripheral, FSH secreting cells and central, LH secreting cells. The reaction to castration of the peripheral and central cells is different. Qualitative changes in the gonadotrophin content of the rat pituitary after castration can be correlated with changes in the relative abundance and state of granulation of these two types of cell.

Purves and Griesbach (1952) have also identified two types of acidophil in the rat. One type reacts by degranulation to marked thyroxine deficiency and may be the source of growth hormone. The other type reacts to castration and oestrogen administration and may secrete prolactin. It is not known in this, or in any other animal, what type of cell is responsible for the production of adrenocorticotrophic hormone.

Clarke (1957) applied the PAS technique to the pituitary of the vole. He studied the adenohypophyses of seven sexually active laboratory bred males, maintained under 15 hours of light daily. Round or oval and angular PAS positive cells could be distinguished. On the basis of the reaction of the granules of these cells with PAS and the morphological similarity of the cells to cells of established function in the rat, they were assumed to be the source of gonadotrophin and thyrotrophin respectively. A second group of seven males, litter

mates of those given long "summer" days, were maintained under the exaggerated winter lighting of 6 hours light per day. As a result they were sexually inhibited. By comparison with their litter mates they had a lower mean testis weight, inhibition of spermatogenesis, poorly developed interstitial cells and smaller penes. This suggested a lowered gonadotrophin output by the pituitary and a lowered androgen output by the testis. Associated with this different degree of sexual development, there was a difference of pituitary cytology. The gonadotrophs of "summer" males contained large amounts of PAS positive material, whereas those of "winter" males were in varying proportion vesiculated and depleted of PAS positive material. The three males with the smallest testes had only vesiculated gonadotrophs in their adenohypophyses. The probability of a lowered output of gonadotrophin by the pituitaries of males under winter lighting suggested that the vesiculated gonadotrophs were inactive cells.

This was a preliminary result. It was based on only a small number of animals, maintained in artificial laboratory conditions. The identification of the round or oval cells as gonadotrophs was based only on their reaction with PAS and their morphological similarity to the gonadotrophs of the rat. It, therefore, seemed desirable to continue the investigation in two main ways:

1. To use the techniques of castration and thyroidectomy to investigate the function of the round or oval and of the angular PAS positive cells of the vole's pituitary. These techniques have been fully developed and investigated in the rat. Removal or inactivation of the target organ alters the pituitary output and also the pituitary content of the particular trophic hormone. The changes in the pituitary content of this hormone can be correlated with changes in a specific cell type. A return to normal pituitary hormone content and to normal pituitary cytology can be produced by replacement therapy, that is by the injection of appropriate doses of androgen or thyroxine (see Griesbach and Purves, 1945; Purves and Griesbach, 1946, 1951b, 1955, 1956).

2. To study the pituitary cytology and the target organs of animals obtained from the field in mid-summer (at the height of the breeding season) and in mid-winter (during the non-breeding period). In order to understand the role of the pituitary in bringing about seasonal change a more complete study of its target organs was necessary. It seemed important that such a study should, in the first instance, be carried out on field animals. A limitation may be imposed upon the interpretation of field data, because of the large number of environmental variables to which field animals are likely to be exposed. However, such a study, in which there is the possibility of investigating the integrated endocrine system,

can uncover endocrinological problems, which can later receive adequately controlled laboratory investigation. It may also indicate the significance in the control of breeding seasons of the preliminary laboratory findings of Clarke (1957).

The study of the cytology of the pituitary of field animals has been supplemented by an experiment using bioassay. This was designed to determine the relative content of gonadotrophin in the pituitaries of field animals in winter and summer.

In presenting the relationship between the pituitary and the gonads, it has so far been assumed that the gonadal changes which occur in relation to the breeding season are secondary to changes in the pituitary. If this is so, then in the winter the gonads should be able to respond to adequate amounts of gonadotrophin. An experiment was performed to test the ability of the gonads of voles in winter to respond to exogenous gonadotrophin.

The reproductive condition of field animals has been assessed by studying the gonads and certain accessory reproductive organs. This has involved weighing organs, to obtain a general indication of their degree of development. More detailed study has been by histological and cytological methods.

There are reasons for supposing that both the adrenal cortex and the thyroid may undergo seasonal change. Clarke

(1957) found that, in addition to the difference in pituitary cytology, there was a difference in the adrenals of voles maintained under "summer" and "winter" lighting. The adrenals of "winter" voles were heavier, apparently due to the presence of a (degenerating) X zone (Chester Jones, 1957) absent from the adrenals of "summer" males. Thyroid activity may increase with decrease in environmental temperature (see Brown-Grant, 1956a), so that a more active winter thyroid might be anticipated. Any seasonal change which does occur, in either the adrenal or the thyroid, may be a direct response to seasonal fluctuations in the conditions of the external environment. It may, however, be a secondary response, associated with the change from a breeding to a non-breeding condition. The adrenal cortex has been shown to have an intimate physiological association with the gonads (Parkes, 1945). Study of possible seasonal changes in the adrenals and thyroid has been by weighing and by examination of their histology and cytology.

In addition it was hoped that the study of adrenals and thyroids could provide some indications of the changes, other than those related to gonad function, which the adenohypophysis may undergo. Their study provided a background to the fuller investigation of the cytology of the adenohypophysis of the vole, which was undertaken using various staining techniques, as well as the PAS method.

Table I

Numbers of Microtus agrestis obtained from the field

	Date of Trapping	Number of males	Number of females
Summer	‡ May 1957	5	5
	June 1959	12	13
	* June 1960	20	-
Winter	‡ January 1957	4	6
	January 1959 6.1.59	4	6
	⁑ 15.1.59	17	7
	* December 1959	8	10
	* January 1960	13	8
	* Dec-Jan. 1961	36	-

‡ Collected by J.R. Clarke.

⁑ Used in an experiment, see p. 51.

* The pituitaries from some or all of the animals in these collections were intended for bioassay.

MATERIALS AND METHODS

A. Trapping of field animals

A total of 174 adult field animals has been used in this study. Table I gives details of the numbers obtained in different years and seasons.

The voles were caught in Longworth small mammal traps (Chitty and Kempson, 1949) on adjacent areas of grassland in Wytham Estate, 4 miles from Oxford. Three days of pre-baiting, when the traps were supplied with oats and hay only, were followed by trapping during the third night. On the third evening the pre-bait catches were released, the oat and hay supply was renewed and carrot was given as a source of water. The traps were emptied early the next morning and re-set, a further catch being made during the day.

The trapped animals were brought into the laboratory, confined in individual cages with food and water and killed with coal gas 1 to 10 hours later. Body weight to the nearest gram was taken, using a spring balance. The body weight of pregnant females includes the weight of foetuses.

Animals trapped overnight spent a maximum of 17 hours in winter, and 14 hours in summer, in the traps. Animals caught during the day were in the traps for as little as 3 hours. When examined in the laboratory before killing, all were in good condition and were apparently unaffected by their confinement.

The method of trapping provided animals in a variety of reproductive states. From the point of view of population studies, however, such trapping may be biassed. Therefore, no estimate can be made of the relative sizes of the populations from which animals were obtained. Nor is it known whether these populations were increasing, at a peak, or declining. Similarly, no estimate of the population age structure can be made from the relative abundance, in the catches, of the broad age classes which have been recognised (see p. 17).

B. Treatment of material

In an initial general examination of the field animals, the following points in particular were noted:

1. The appearance of the testes in winter males, whether they were shrunken and wrinkled or smooth in outline. This is of help in distinguishing regressed from inhibited males (see Brambell and Hall, 1939 and p.18-19).

2. The degree of mammary development in summer females. This has been used in distinguishing primipara from multipara (p. 26). Many summer females were obviously pregnant. In those females in which foetuses were not evident in the uterus, no attempt was made to detect the presence of tubal ova or unimplanted blastocysts. It is, therefore, not possible to say whether these females were or were not pregnant.

Paired adrenals were weighed fresh on a torsion balance to 0.1 mg. Paired testes were also weighed fresh to 0.1 mg., on a torsion balance if less than 100 mg., on a beam balance if more than 100 mg.

Thyroids and pituitaries were exposed, but left in situ, and the whole animals were placed in fixative about 15 minutes after death.

Formal sublimate was the chief fixative. Its use results in good fixation and brilliant staining of the vole pituitary, and facilitates comparison with the extensive work of Purves and Griesbach on the pituitary of the rat. They also used formal sublimate fixation.

After about 12 hours in fixative, the material was washed in tap water and transferred to 70% alcohol, before further dissection. Then thyroids, seminal vesicles, ventral prostates, the uteri of winter females and both ovaries were removed, blotted on filter paper and weighed on a torsion balance to 0.1 mg. Pituitaries were removed, but, to avoid damage which would interfere with critical cytological study, they were not weighed. A binocular microscope was used in dissection.

Bouin's fixative has also been used for organs other than pituitaries.

In some males and females the right adrenal, and the right testis of males, was fixed in formal calcium to preserve lipids. The corresponding left organ was fixed in formal sublimate or

in Bouin's fixative.

The formulae of the fixatives used are given in Appendix I.

Storage of formal sublimate fixed material was in 70% alcohol. The Bouin and formal calcium fixed material was stored in fixative.

Organs fixed in formal sublimate and Bouin's fixative were embedded in 54°C melting point wax, using either Cedar Wood Oil or benzene as antemedium.

Adrenals, thyroids, testes, seminal vesicles, ventral prostates, ovaries and uteri were sectioned transversely at 5 μ. The routine stain for all these organs was Ehrlich's haematoxylin and eosin. Some adrenal sections were also stained by the McManus periodic acid-Schiff (PAS) technique (Appendix II). This enables the connective tissue framework of the gland to be studied.

Pituitaries were sectioned either sagittally or coronally at 5 μ with some 2 and 3 μ sections also from each pituitary. One mid-line section of each pituitary was stained by the McManus periodic acid-Schiff technique, using the method of Purves and Griesbach 1951b. Every 10th section of a number of pituitaries was also stained in this way, in order to study the distribution of the different cell types. In further sections stained by PAS, a counterstain of 2% orange G in 5% phosphotungstic acid (Pearse, 1954) was used to study acidophils.

Table II. Numbers of field animals on which histological observations are based

Season	Sex	Pituitary	Thyroid	Adrenal		Ovary	Testis		Uterus	Seminal vesicle	Ventral prostate
				P	G		P	G			
Summer	♂	16	5	14	3	-	8	3	-	5	5
	♀	18	6	14	4	5	-	-	0	-	-
Winter (excluding 1958-59)	♂	9	5	30	8	-	45	8	-	7	5
	♀	12	4	10	8	5	-	-	6	-	-
Winter of 1958-59	♂	11	0	12	6	-	12	6	-	0	0
	♀	9	0	9	4	9	-	-	0	-	-

P. paraffin section

G. frozen section of gelatine embedded material examined for lipids.

Some 3 μ sections from both winter and summer pituitaries were stained by Crossmon's modification of Mallory's trichrome stain (Crossmon, 1937). Pituitary sections from each season were also stained with aldehyde fuchsin (AF) (Gomori, 1950). In order to obtain the best results with the vole's pituitary, it has been found necessary, in each case, to modify the time of exposure to the various stains and reagents. Details of all pituitary staining methods are given in Appendix II.

The adrenals and testes fixed in formal calcium were embedded in gelatine, frozen sections were cut transversely at 10 μ and sections from the middle of the organ were coloured with Sudan Black.

Table II shows the numbers of animals on which the histological observations are based. The emphasis has been on the pituitary and the adrenal. The thyroids of only some animals have been examined histologically. Observations on sections of the gonads of 8 summer males and of 5 winter and 5 summer females have confirmed the descriptions of previous investigators. Examination of the testes of males in winter, however, raised a number of problems and winter testes have, therefore, received a comprehensive histological study. Sections have also been cut of some seminal vesicles and ventral prostates in each season, and of the uteri of females in winter, though, in general, weight gives an adequate guide to the degree of development of these organs (see p.22, 26).

C. Criteria for determining the approximate age of field animals.

The age of field animals could not be accurately determined, but it has been found possible to divide animals into broad age classes, containing animals which differ in reproductive experience.

It seems likely that, in an animal which is sexually mature but not senile, past events in its reproductive life, rather than age as such, will be important in influencing its endocrine organs. Endocrine differences in the reproductive sub-groups established have, therefore, been investigated.

In this section the literature relevant to the approximate determination of age in field animals is presented.

All the animals had adult fur. Laboratory experience suggests that they were, therefore, at least 30 to 40 days old and probably potentially capable of reproduction. In the laboratory males mature at about 6 weeks, females at about 3 weeks (Leslie and Ranson, 1940).

Voles have been studied in the field by Baker and Ranson (1933), Brambell and Hall (1939) and Chitty (1952). Their studies indicate that the breeding season of voles in Britain extends from February or March to September or October. Animals born early in one year breed in the same year, are sexually inactive in the winter and breed again the following spring. Animals born later in the year do not breed until

spring. Survival for two winters is probably a rare occurrence.

Animals caught in January will, therefore, be expected to fall into two broad age classes:

1. Those which have been sexually active and are between 6 and 9 months old.

2. Those which were born at the end of the breeding season, did not breed and are about 3 to 6 months old.

Animals caught in June should also be divisible into two age classes:

1. Animals which have over-wintered and are 8 or more months old.

2. Young of the year which are about 2 to 3 months old.

Brambell and Hall (1939) give criteria for distinguishing mature and immature animals in winter:

1. The testes of immature males are plump and regular in outline. The tunica albuginea is thin. Seminiferous tubules are closely packed together and spermatogonia lie away from their walls.

2. The testes of mature animals have a collapsed appearance. The tunica albuginea is a thick structure. The seminiferous tubules are loosely arranged, with relatively enormous inter-tubular spaces, and spermatogonia lie against their walls.

3. The uteri of parous females show fibrosed blood vessels, which are absent from the uteri of females which have not been pregnant.

These criteria have been found to be only partially satisfactory. They are critically discussed later (p.28 ff).

The terms "mature" and "immature" or "prepubertal", used by Brambell and Hall (1939) to refer to males in winter, are here replaced by the terms "regressed" and "inhibited". A regressed male is one which was sexually active in the preceding summer, but whose testes regressed from an active to an inactive condition at the end of the breeding season, with the onset of unfavourable external conditions. An inhibited male is one which was born late in the breeding season and has never been sexually active. Although of an age to be sexually active, its sexual development has been inhibited by the unfavourable conditions of the environment. The use of these special terms emphasises the nature of winter sexual inactivity. Both groups of animals are somatically mature. They have adult fur. It is probable that, endocrinologically, inhibited, as well as regressed, males are quite different from juvenile animals, less than 30 to 40 days old, which can strictly speaking, be said to be immature or prepubertal. Similar considerations apply to the winter sexual inactivity of females, both non-parous and parous.

Chitty (1952) uses body weight to distinguish old and young animals. 22.25 g. was chosen as a suitable weight, following the collection of body weight data and the study of marked individuals, during an extensive study of vole populations

in Montgomeryshire. He notes that old animals, which have lost weight, and some rapidly growing animals and pregnant females may be wrongly classified by this method, but in general it is considered to be quite accurate.

CHAPTER I. THE REPRODUCTIVE TRACT

A. Seasonal Changes in the Reproductive Tract

In general, the voles show the expected winter-summer contrast in reproductive condition. However, variations are seen to occur between years, as well as between seasons. In particular the winter of 1958-1959 was most unusual.

(1) Weight data

For each sex in each year and season the frequency distributions of body and organ weights were prepared. Arbitrary weight classes were used. For example body weight was considered in 2 g. groups, that is 12.0 - 13.9 g., 14.0 - 15.9 g. and so on.

These frequency distributions showed:

1. That the males in the 1958-59 winter should be considered separately. The frequency distributions of testis and seminal vesicle weights in this winter were quite different from those in other winters.
2. That between years comparisons for summer males were necessary. The frequency distributions of seminal vesicle and ventral prostate weights was different in the two summers for which data on the weight of accessory organs was available. However, in the comparisons with winter males, all the data on summer males have been pooled.
3. That for females variation between years was not marked.

Table III. Mean body and organ weights for

	Summer	Winter excluding 1958-59	Winter regressed
Body weight (g)	34.35± 0.84	19.31± 0.32	22.78± 2.18
Testes (mg)	540.60±76.35	16.13± 2.92	40.41±17.30
Seminal Vesicles (mg)	238.02±19.83	1.03± 0.19	2.89± 0.93
Ventral Prostates (mg)	39.54± 2.69	3.79± 0.60	6.23± 2.35
Adrenal (mg)	7.48± 0.22	5.69± 0.19	6.40± 0.77
Thyroid (mg)	2.38± 0.13	1.55± 0.06	2.04± 0.32

			Size of
Body weight	37	61	9
Testes	37	61	9
Seminal Vesicles	31	57	8
Ventral Prostates	26	52	8
Adrenal	37	61	9
Thyroid	28	55	8

Details of tests of statistical

males. ± Standard Errors

Winter inhibited	2-3 month Laboratory stock	All 1958-59 Winter	1958-59 Winter Testes > 100mg	1958-59 Winter Testes < 100mg
18.58± 0.32	24.61± 1.27	20.17± 1.08	22.60± 2.10	18.43± 0.57
11.50± 1.12	332.31±28.54	142.54±48.90	291.26±80.93	42.89± 3.58
0.62± 0.08	81.55±13.93	18.00± 6.56	41.12± 7.40	1.49± 0.22
2.10± 0.42	21.16± 5.59	-	-	-
5.76± 0.28	5.32± 0.34	6.62± 0.37	6.00± 0.45	7.13± 1.27
1.42± 0.06	1.82± 0.11	1.78± 0.10	1.0) 1.8) 1.40	1.5) 1.6) 1.8) 1.8) 1.67
Samples				
31	13	12	5	7
31	13	12	5	7
28	13	12	5	7
26	13	-	-	-
31	13	10	5	6
26	13	6	2	4

significance are given in Appendix III

4. That in each winter the uterine weights of females fell into two distinct classes.

5. That there is no criterion of weight by which males can be divided into two classes in winter.

Tables III, IV and V give summaries of the data collected on weights of organs and on body weight. Details of the tests of statistical significance are given in Appendix III.

In this chapter the data on the weight of the body, the gonads and accessory reproductive organs is considered.

a. Males (excluding those caught in the winter of 1958-59)

The mean body weight of summer males is almost double that of winter males (Table III).

The mean weight of testes in summer is over 30 times greater than their mean weight in winter. Brambell and Hall (1939) give 20 mg. as the maximum weight of winter testes. Although the majority of testes do weigh less than this, a few males have been obtained in each winter with greater testis weights.

The weights of seminal vesicles and ventral prostates also show marked variation with season, being higher in summer. However, although the mean weights of the ventral prostates in summer and winter show a highly significant difference, they do not differ so strikingly as the mean weights of summer and winter seminal vesicles. There is a more than 200 fold difference between summer and winter mean seminal vesicle weights; for the ventral prostate

the difference is only 10 fold. While in summer the mean weight of the seminal vesicles is about 6 times that of the ventral prostates, in winter ventral prostates are almost 4 times heavier than seminal vesicles.

The data on 2 to 3 month old laboratory stock is included for comparison with the winter males. The killing of these animals of known age and the weighing of their organs was carried out as described for field animals, p.12,14. All the field males in winter were at least 3 months old, but their degree of sexual development is much less than that of 2 to 3 month old males, raised in the warm, light conditions of the laboratory.

Males in winter can be divided into two groups on the basis of the structure of the tunica albuginea. As will be discussed later p. 29 , it is probable that this criterion enables regressed and inhibited males to be identified in winter. Of the 45 winter testes, from years other than 1958-59, which were examined histologically, 40 could be put into one or other of these categories. A comparison of mean weights in these two sub-groups (Table III) shows that only in the case of the seminal vesicles is there a statistically significant difference between the regressed and inhibited animals. The mean weights of the body, the testes and the ventral prostates are higher for regressed than for inhibited males. The difference in body weight reaches the borderline of statistical significance. The difference in testis and ventral prostate weights is not

Table IV. Comparison between summer males: mean organ weights ± standard errors

	1957	1959	1960 (Assay)	
Body weight (g.)	33.60 ± 2.98	36.00 ± 1.64	33.55 ± 2.62	n.s.
Testes (mg.)	528.00 ± 43.18	520.83 ± 31.78	555.50 ± 16.82	n.s.
Seminal vesicles (mg.)	-	330.78 ± 31.31	189.90 ± 15.36	$P < 0.001$
Ventral prostates (mg.)	-	46.68 ± 3.10	34.13 ± 3.56	P 0.02-0.01
Adrenal (mg.)	8.22 ± 0.97	7.49 ± 0.38	7.30 ± 0.85	n.s.
Thyroid (mg.)	-	2.76 ± 0.20	2.10 ± 0.12	P 0.01-0.001

	Size of samples		
Body weight	5	12	20
Testes	5	12	20
Seminal vesicles	-	12	15
Ventral prostates	-	12	14
Adrenal	5	12	20
Thyroid	-	12	16

statistically significant. Although mean weights differ considerably, both these organs are subject to very wide variation in weight in regressed males.

Table IV shows that the mean weights of seminal vesicles and ventral prostates of voles caught in the summer of 1959 are heavier than in the summer of 1960. These differences are statistically significant. There is no significant difference in mean testis weight in the two summers.

b. Males caught in the winter of 1958-59

The males caught in this winter were in a number of ways exceptional. The collection consisted of 21 males and 12 females. 4 males and 5 females were caught on January 6th. 1959 and were treated as previously described, p.12ff. The 17 males and 7 females caught on January 15th. 1959 were used in an experiment (see p.51ff) and only the controls from the experiment have been included in the statistical treatment of data. The controls received rather different treatment from the other field animals. They were maintained in cages kept in the open air and were handled for short periods daily. Six days intervened between their being captured and killed. However, the close similarity between them and the animals caught 9 days previously was considered to justify their inclusion.

The mean weights of testes in this winter was about 9 times heavier than in other winters, though still considerably

Table V. Mean body and organ weights for females ± standard errors

	Summer	Winter	Summer primipara	Winter multipara	Winter Parous	Winter Non-parous
Body weight (g.)	24.56±1.41	18.88±0.65	19.00±0.95	28.09±1.41	20.08±1.86	16.86±0.81
Ovary (mg.)	4.98±0.35	0.82±0.10	4.60±0.26	5.21±0.55	1.12±0.13	0.47±0.06
Adrenal (mg.)	9.99±0.55	6.21±0.36	9.31±0.87	10.43±0.72	7.75±0.51	5.25±0.30
Uterus (mg.)	-	-	-	-	13.30±0.73	2.79±0.11
Thyroid (mg.)	1.65±0.15	1.63±0.13	1.58±0.26	1.80±0.15	2.08±0.12	1.13±0.10
			Size of samples			
Body weight	18	30	7	11	13	11
Ovary	13	24	5	8	13	11
Adrenal	18	30	7	11	13	11
Uterus	-	-	-	-	13	11
Thyroid	11	23	5	6	12	11

Details of tests of statistical significance are given in Appendix III.

lighter than the summer mean weight. Seminal vesicles were also unusually heavy, Table III.

Brambell and Hall (1939) found that 100 mg. was a good dividing line between testes with and without sperm. The males of the 1958-59 winter were, therefore, considered in two groups:

1. Those with testes weighing more than 100 mg.
2. Those with testes weighing less than 100 mg.

There is a statistically significant difference between the mean seminal vesicle weight of the two groups. It is higher in group 1.

In group 1 the mean body weight and mean seminal vesicle weight are much higher than in other winters, though still well below their summer mean values.

In group 2 the testes are about the same weight as those of regressed males in other winters, but mean body weight and seminal vesicle weight are nearer the values for inhibited males.

c. Females

The body weight of females in summer is higher than in winter (Table V). The difference is statistically highly significant. Many summer females were pregnant and their body weight includes the weight of foetuses. It is, therefore, not possible to say whether there is any seasonal difference in female body weight other than that produced by pregnancy.

There is a five fold difference in ovarian weight between

summer and winter.

It is suggested that parous and non-parous females can be distinguished in winter on the basis of uterine weight. It has been noted that the weights of uteri fall into two quite distinct classes in winter. The difference in histological appearance of uteri in the two groups has already been described by Brambell and Hall (1939) as a criterion for distinguishing parous and non-parous females (see p. 18).

Once a separation on the basis of uterine weight has been made, it becomes evident that mean ovarian weights differ in the two groups; the difference is statistically highly significant. In both groups, parous and non-parous, the mean ovarian weight is significantly lighter than in summer females, however.

There is also a difference between the mean body weights of parous and non-parous females, but it is not statistically significant.

Of fourteen summer females which were clearly pregnant, seven showed no mammary gland development. They also had lower body weights than those pregnant females which appeared to be lactating. These lighter females without mammary gland development were very probably in their first pregnancy. They have a lower mean ovarian weight than multipara, but the difference is not statistically significant.

The females in the 1958-59 winter showed no statistically significant differences in the weights of their organs when compared with females in other winters and so have not been listed separately in Table V.

(2) Histology

A detailed description of the anatomy and histology of the reproductive tract of Microtus arvalis has been given by Delost (1955a). The reproductive tract of Microtus agrestis is similar in its structure to that of Microtus arvalis.

a. Summer males

The testes show all stages of spermatogenesis. Seminiferous tubules are about 140 to 220 μ in diameter. The interstitial cells are conspicuous. They have vesicular nuclei and cytoplasm filled with lipid droplets (Plate I, Figs. 1, 2).

The seminal vesicles are filled with an eosinophil secretion. The epithelium which lines them consists of high columnar cells. Secretion granules are present in the cytoplasm between the oval nucleus and the free border of the cells (Plate I, Fig. 3).

Ventral prostate follicles are distended by a homogeneous eosinophil secretion and lined by columnar epithelium. Fine vacuoles may be present in the distal area of the epithelial cells (Plate I , Fig. 4).

b. Winter males (excluding those of 1958-59)

Seminiferous tubules are about 40 to 80 μ in diameter. In the majority of testes they contain only spermatogonia and Sertoli cells (Plate II , Fig. 1,2). However, in some testes from each winter, signs of mitotic activity in spermatogonia and the presence of occasional primary spermatocytes has been noted. The interstitial cells have darkly staining, misshapen nuclei and scanty cytoplasm, from which lipid droplets are absent (Plate II, Fig. 3).

As mentioned previously (p. 18) Brambell and Hall (1939) give four criteria for distinguishing mature (regressed) from prepubertal (inhibited) males in winter. However, only one of these, namely the structure of the tunica albuginea, seems reliable. Rowlands (1936) gives the same 4 criteria for Clethrionomys glareolus.

In particular, the loose arrangement of tubules with relatively enormous inter-tubular spaces, which is described for mature animals of both species in winter and which is illustrated in Rowland's paper, is almost certainly an artifact, produced in preparing material for microscopic examination. Loose arrangement of tubules may appear in paraffin sections of testes obtained from animals at any time of year and is never seen in frozen sections of gelatine embedded material. It is, however, possible that such an appearance is more

easily produced in testes which have regressed from a summer breeding condition.

The different positions of spermatogonia described (see p. 18) could not be detected.

However, males in winter can be divided into two groups on the basis of the structure of the tunica albuginea. In one group it is thin; in the other group it is a much thicker structure, with a characteristic wavy appearance in the connective tissue, particularly at the outer border (Plate II, Figs. 1, 2). One male in the 1956-57 winter collection and 8 in the 1960-61 collection showed the latter type of tunica albuginea. In only 2 animals was this associated with a flabby appearance on dissection and gross irregularity of the outline of sections, one of Brambell and Hall's criteria. It seems reasonable to suppose that a thin tunica albuginea is a characteristic of inhibited males, while a thick tunica, which has the appearance of having undergone contraction, is a characteristic of males with regressed testes.

As has previously been mentioned, males differ from females in that there is no criterion of weight on which they can be divided into distinct classes in winter. If the structure of the tunica albuginea is accepted as a reliable criterion, it is found that in only four of nine animals classed as regressed are the body, testis and accessory organ weights outside the range for inhibited males. Even when the

distinction has been made, on the basis of the structure of the tunica albuginea, only the seminal vesicles show a statistically significant difference in mean weight in the two groups. The mean weights of the body, testis and ventral prostates are, however, higher in regressed males (see p. 23).

A further 5 males from the 1960-61 collection could not be classified as regressed or inhibited. 2 males were sexually developed: one had sperm in its testis, another had spermatids. 3 males had testes with only spermatogonia and Sertoli cells, but with a tunica albuginea neither thin nor thick, but of intermediate structure.

The lack of activity in the testes is reflected in the structure of both the seminal vesicles and the ventral prostates.

The seminal vesicles, in both regressed and inhibited males are devoid of secretion. The epithelium lining them is markedly reduced in height; the cytoplasm is so scanty that nuclei almost fill the cells. The muscle layers tend to be thicker in regressed than in inhibited males. (Plate III, Figs. 1, 2). This is probably the source of the higher mean weight of seminal vesicles in the former. Table III.

Similarly the follicles of the ventral prostate are not distended by a homogeneous eosinophil secretion. Instead, they contain only a small amount of pale staining granular secretion; the lining epithelium is folded. However, the

cells of the epithelium are high columnar, appearing even higher than in summer (Plate II, Fig. 4). It has been noted earlier (p. 22) that the seasonal weight change in the ventral prostates is statistically highly significant, but that it is not so great as that in the seminal vesicles. The involution which the ventral prostates undergo in a sexually mature male is not so extensive or complete as that undergone by the seminal vesicles.

Delost (1955b) has found in Microtus arvalis a marked stimulation of the ventral prostate, of the epithelium of the vas deferens and the occurrence of secretion in the cauda epididymis of winter males, despite atrophy of the other male reproductive organs. In the female he describes tissue homologous with the ventral prostate which is also stimulated. Delost gives no data on the weight of the ventral prostate, but it is stated to be almost as large in winter as in summer.

c. Summer females

Summer females may be considered in two groups:

1. Primipara

As mentioned previously seven of the fourteen pregnant females obtained were judged, by absence of mammary development and low body weight, to be in their first pregnancy.

2. Multipara

Eleven lactating females were obtained. Of these 7 were pregnant and 4 not obviously pregnant. There were no

significant differences in body or ovarian weight between the pregnant and non-pregnant multipara.

No virgin females were obtained.

The ovaries of all summer females contain, besides follicles in different stages of development, corpora lutea of pregnancy or lactation.

The maximum follicle diameter recorded was 550 μ, for a follicle which had three to four rows of granulosa cells lining the antrum. Brambell and Hall (1939) give the mean diameters of groups of large follicles from the ovaries of two females in pro-oestrus as 610 and 652 μ, respectively. This may be taken as a rough guide to the size of follicles at ovulation.

d. Winter females

In December and January no pregnant females were caught. The ovaries of winter females frequently contain follicles with antra. The maximum follicular diameter recorded, 340 μ, is, however, well below the estimated size of follicles at ovulation.

The observation of Brambell and Hall (1939), that in winter parous and non-parous females can be distinguished by histological examination of the uterus, has been confirmed. The uteri of parous females show fibrosed blood vessels. Further it has been found that in winter a parous uterus can be distinguished from a non-parous one by weighing (p. 26).

e. The 1958-59 winter collection

The unusually high testis and seminal vesicle weights recorded in this winter have already been commented upon (p. 24).

The testes weighing over 100 mg. all contain sperm (Plate III, Fig. 3.). In 4 animals sperm are present in the majority of seminiferous tubules and in one animal in a few tubules. The degree of development of the interstitial cells varies, from cells with pycnotic nuclei and scanty cytoplasm containing no lipid droplets, to cells with vesicular nuclei and more abundant cytoplasm, containing lipid droplets. However, in none of these animals are the interstitial cells as conspicuous, well developed or apparently as numerous as in a summer male. The mean seminal vesicle weight is well below the summer mean value, Table III.

Of the 7 males with testes weighing less than 100 mg., 6 show spermatids and 1 secondary spermatocytes. All show abundant mitotic activity in the seminiferous epithelium. None of the males caught in this winter show only spermatogonia and Sertoli cells in the testes. The interstitial cells are moderately well developed, with more cytoplasm and less darkly staining nuclei than in other winters. There are, however, few lipid droplets in the cytoplasm of the interstitial cells. (Plate III, Fig. 4).

No obviously pregnant females were caught in this winter and examination of serial sections of ovaries showed no corpora

lutea.

In the 1960-61 winter one male was caught with sperm in its testis and another with spermatids.

(3) Discussion

a. Age determination

Criteria have been described which, it is considered, enable animals in both the winter and the summer collections to be divided into sub-groups which differ in reproductive history and also, broadly, in age (see p. 17 ff, 26, 28 ff.).

Possible endocrine differences between these sub-groups have been investigated. It seems likely that in an animal which is sexually mature, but not senile, past events in its reproductive life, rather than its precise age, will be important in influencing its endocrine organs.

The division of winter females, by weight and histology of the uterus, is clear. The majority of winter males examined could also be assigned to one or other class on the basis of the structure of the tunica albuginea. There were, however, some males which could not be classified with certainty. Perhaps their sexual development had been arrested when partially completed. Males become potent at about 6 weeks of age (Leslie and Ranson, 1940). Males of Microtus arvalis are able to reproduce at a similar age (40 days), increase in testis weight beginning at 7 days (Delost, 1956a). The process of

attaining sexual maturity is thus spread over a period of weeks, and there may be animals in a winter population in which gonad maturation had proceeded part way, before unfavourable external conditions intervened and regression set in.

For females, also, there is a process of gonad maturation, proceeding gradually and culminating in the establishment of oestrus cycles at about 3 weeks of age. Virgin females may undergo a number of dioestrus cycles, before becoming pregnant (Brambell and Hall, 1939). However, it is the change in the uterus, which results from having experienced pregnancy, which enables females in winter to be divided into two distinct groups: parous and non-parous. It is not the establishment of oestrus cycles as such, or the processes leading to this. It is these processes which are comparable to puberty changes in the male.

In the summer classification into reproductive and age classes is more difficult. Females can be identified as primipara or multipara. Animals in their first pregnancy in May - June will almost certainly be young of the year, but the group of multipara probably includes both young of the year and animals which have over-wintered. 5 of the 11 multipara have body weights over 30 g. These are almost certainly females which have over-wintered. Laboratory experience indicates that it is very unlikely that a 2 to 3 month old female would have

reached such a high body weight even if pregnant. One female with a body weight of 22 g. is very probably young. 5 females with body weights of 24 to 26 g. cannot be assigned with certainty to either age class.

The only way in which the age of males in summer can be assessed is by body weight. As mentioned previously, Chitty (1952) used a body weight of 22.25 g. to separate old from young animals. In particular he notes that throughout one year young males did not increase beyond 22.2 g. in weight, whereas adult males seldom weighed less than 22.3 g. after May. This may be compared with 25 g., the mean body weight of 2 to 3 month old laboratory animals, Table III. Although it is evident that caution should be used in transferring the results obtained in one population to another, it seems unlikely, as in females, that a 2 to 3 month old field animal would weigh as much as 30 g. Only one male had a body weight less than 22.25 g. and only 7 of 37 males had body weights less than 30 g. It, therefore, seems that the vast majority of males which have been caught in summer are old animals which have over-wintered.

b. The Length of the Breeding Season

In the summer Microtus agrestis shows a high level of sexual activity. All the females obtained were pregnant or nursing litters. Males had testes packed with sperm. The lower mean weights of ventral prostates and, in particular, seminal vesicles in the summer of 1960 as compared with 1959,

perhaps indicates a between year variation in the level of fertility. However, it is clear that in 1960 males were sexually active and litters are known to have been conceived.

In most winters voles are sexually inactive. From November to the beginning of February males have testes with only spermatogonia and Sertoli cells in the tubules and with a poorly developed interstitium (Baker and Ranson, 1933; Brambell and Hall, 1939). The seminal vesicles are atrophic. The ventral prostate, however, appears to be stimulated and this will be discussed further in connection with the castration experiments. During the same period, females are in anoestrum. Ovarian follicles remain relatively small, but may show antra. Oocyte development can proceed up to the stage of antrum formation in the absence of pituitary gonadotrophins (Hisaw, 1947). The presence of follicles with antra in winter females might, therefore, indicate a low level of gonadotrophin secretion, either sufficient to produce development to this stage or to maintain follicles whose growth was arrested by a drop in gonadotrophin secretion, following the onset of unfavourable external conditions in autumn.

Winter breeding in Microtus agrestis has not been reported in Britain, but Raynaud (1951) reports winter breeding in this species in southern France.

Closely related species quite frequently show winter breeding; in January 1959 pregnant Clethrionomys glareolus

were obtained, while trapping for Microtus agrestis. Baker (1930) reports sporadic winter breeding in both Apodemus sylvaticus and Clethrionomys glareolus. Steven (1957) found that in a colony of Clethrionomys glareolus maintained in the open air litters were born in every winter.

It is not known whether breeding in Microtus continued throughout the 1958-59 winter. No pregnant females were obtained or females with corpora lutea, but there were males with sperm in their testes in the January catches. One non-parous female weighed only 11.5 g. It had adult fur, but its low body weight indicates that it was a very young animal. A parous female of 25 g. was unique among the winter females trapped, in that its mammae were still well developed. These facts suggest that breeding had continued until late in the year.

The group of males with testes which weighed less than 100 mg. and which contained secondary spermatocytes and spermatids were either:

1. Males which had been sexually mature and were undergoing regression, or
2. Young males beginning their sexual development.

If the males had been undergoing regression, it would have been anticipated that they would have a high seminal vesicle weight, since changes in accessory organs lag behind changes in the gonads (see, for example, Moore et al., 1934, for the

13-lined ground squirrel). In fact the seminal vesicles weigh only 1.0 to 2.5 mg. On the other hand, a low seminal vesicle weight is compatible with the suggestion that the males were beginning their sexual development. Again changes in the gonads precede changes in the accessory organs. Delost (1956a) finds that in Microtus arvalis growth of the accessory organs lags behind maturation changes in the seminiferous epithelium. The first sperm may be present as early as 35 days, when growth of the accessory reproductive organs is only just beginning. The low body weight of these males, below 20 g., and the presence of active mitoses in the seminiferous epithelium also supports the view that they were beginning their sexual development.

There is no evidence available to explain satisfactorily the variations between years in the state of the reproductive system: namely, the different mean weights of male accessory organs in the summers of 1959 and 1960; the gonadal development of males in the winter of 1958-59. The occurrence of sexually developed voles in the winter of 1958-59 is of special interest. In December and January gonads are generally inactive.

Light and possibly temperature influence the growth and activity of gonads in the vole (Baker and Ranson, 1932a, 1932b; Kennedy, 1962). It takes some time (weeks or even months) for the gonads of laboratory voles, maintained in experimental light and temperature environments, to regress or to become

Table VI. Meteorological data

Mean monthly temperature ± SE (°F) for period September-December inclusive

1956	1958	1959	1960
47.9±3.6	48.8±4.1	50.2±3.9	48.0±3.5

Mean hours of sunshine per month ± SE for period September-December inclusive

1956	1958	1959	1960
76.7±21.1	73.3±20.0	118.5±38.2	77.9±15.5

Mean monthly temperature ± SE(°F) for period February-May inclusive

1959	1960
47.1±3.4	46.7±3.4

Mean hours of sunshine per month ± SE for period February-May inclusive

1959	1960
137.3±35.2	126.9±27.2

Data derived from records of the Radcliffe Meteorological Station, Oxford and measured at a site 4 miles from habitat from which voles were collected.

active. Therefore, had climatic factors been responsible for the prolongation of sexual activity into the winter, it would seem reasonable to make between-year comparisons of, for example, temperature and hours of sunshine, during the 4 months preceding the dates of trapping. Baker and Ranson (1933) found a general correlation between hours of sunshine per month and the breeding condition of voles.

It is clear from Table VI that an explanation for the prolongation into the winter of gonad activity cannot be provided by the temperature and light conditions prevailing in the preceding autumn. The period September to December 1958 was neither the coldest nor the warmest of the series. There were fewer hours of sunshine in this period in 1958 than in other years, though the difference is very small. If having less sunshine influences vole reproduction, conditions in 1958 would, if anything, have hastened regression or enhanced inhibition of gonads.

The existence of apparently active testes in the winter of 1958-59 cannot be explained, then, on the basis of unusual climatic conditions.

Similarly, climatic conditions of sunshine and temperature (Table VI) cannot explain the different accessory organ weights of fecund voles in the summer of 1959 and 1960.

The possibility of nutritional differences between winters

and between summers cannot be excluded as an explanation for the between-year differences in reproduction.

Chitty (1952) found appreciable differences in the length of vole breeding seasons in different years which he attributed to differences in population size or phase. Steven (1957) found that in a colony of bank voles, Clethrionomys glareolus, maintained in the open air, breeding continued throughout the year. There were, however, fewer litters born in December than in the spring. He could not correlate the amount of winter breeding in any year with any obvious climatic variable. He thought it was more likely to be related to the age structure and fecundity of the animals forming the colony.

A similar type of factor perhaps explains the occurrence of two males with developed testes in the 1960-61 winter collection, which otherwise consisted of males showing no signs of sexual development.

Perhaps, then, social or demographic factors may, on occasion, be able to over-ride or modify the effects of light and temperature. Clearly, light and temperature are not the only external factors controlling the sexual cycle of the vole.

B. The Effects of Castration on Seminal Vesicles and Ventral Prostates.

The histological appearance of the testicular interstitial cells and the generally atrophic state of the reproductive tract in sexually inactive, winter males suggests a low level or an absence of circulating androgen. Castration of sexually active males removes the primary sources of androgen. Similarities between winter males and castrated males may, therefore, be expected.

The castration experiments have been performed primarily as a means of identifying the pituitary cells responsible for the production of gonadotrophic hormones, but seminal vesicles and ventral prostates of male castrates were available for comparison with those of male field animals in winter.

(1) Methods

The testes have been successfully removed from 11 laboratory bred, sexually mature male voles. They were 11 to 22 weeks old when castrated and all survived for the full period of the experiment.

For each castrate there was a control, in 9 cases a litter mate, in 3 a male of comparable age.

The animal to be castrated was chosen at random from the pair. It was anaesthetized with Nembutal, 0.15 ml. of a 1 to 9 dilution in 0.9% sterile saline being given intraperitoneally. About 10 minutes later, anaesthesia was

cautiously completed with ether, a cone of perforated zinc containing ether soaked cotton wool being placed over the nose of the vole.

Delost (1956b) comments on the difficulties involved in anaesthetizing Microtus arvalis. He used ether anaesthesia alone.

A combination of Nembutal and ether has been found satisfactory, but care must be taken. The susceptibility to Nembutal varies widely and if large doses of ether are necessary, respiratory difficulties may occur. In these circumstances the ether is better administered in a desiccator, with ether soaked cotton wool in the bottom and the vole placed on a perforated zinc sheet above it. Careful watch must be kept on its respiratory movements. 3 voles died as a result of the administration of anaesthetic.

A transverse incision was found to be most convenient for the removal of testes. The spermatic cords were ligatured and the testes carefully removed. The wound was sutured with a continuous thread.

When the operation was completed, the animal was placed in a prepared cage in a bed of cotton wool, as temperature regulation is poor in anaesthetized animals. Additional warmth was supplied by directing a 60 watt bulb onto a sheet of copper on top of the cage, thus giving heat without bright

Table VII. Effects of castration: male organ weights

	Number of animals	Mean weights of specified organs		Mean difference ± S.E.	P
		Control	Experimental		
Initial body weight (g)	8	30.25	30.37	0.12±1.80	n.s.
Final body weight (g)	8	30.75	33.62	2.87±2.74	n.s.
Testes (mg.)	9	350	365	15 ± 27	n.s.
Seminal vesicles (mg.)	8	171.00	50.29	120.71±29.92	0.01-0.001
Ventral prostates (mg.)	8	26.67	6.56	20.11± 6.57	0.02-0.01
Adrenals (mg.)	9	5.87	9.00	3.13± 0.87	0.01-0.001
Thyroid (mg.)	9	2.19	2.08	0.11± 0.35	n.s.

Comparison of Initial and Final body weight of experimental animals

No.	Initial weight	Final weight	Mean difference ± S.E.	P
8	30.37	33.62	3.25 ± 3.22	n.s.

light. Consciousness was usually recovered within 1 hour of giving the Nembutal injection and normal activity was resumed within another hour. The wounds healed well and survival rates were high; only 2 animals died post-operatively.

Both castrated and control animals were maintained, one animal to a cage, at 18-21°C and with 16 hours of light per day. They had unlimited water, hay, and oats, plus carrot supplied twice weekly. Body weights were recorded once a week.

The animals were killed with coal gas at 7, 14, 21, 28, 49, 56, 84 and 112 days after castration. Fresh weights of testes and fixed weights of seminal vesicles and ventral prostates were taken. Testes were fixed in either formal sublimate or Bouin's fixative. The corpse was fixed in formal sublimate. Organs were prepared for histological examination as previously described, p. 14.

(2) Results

a. Weight data - Table VII

Litter mate comparisons only have been made in the calculations. This means that the number of animals appearing in the tables is less than the total number in the experiment.

The castrated animals show a slight increase in mean body weight, both over their own initial mean weight and over that

of the controls, who show no such increase. The differences are not statistically significant.

(i) Seminal vesicles

There is a highly significant drop in the mean weight of seminal vesicles. 28 days after castration they are about one third of their original weight. By 56 days they have regressed still further and their weight appears to be approaching a lower limiting value. The lowest seminal vesicle weight recorded was 2.4 mg., in an animal castrated for 112 days. This is of the same order of magnitude as the seminal vesicle weight of a regressed male in winter (Table III).

(ii) Ventral prostates

There is also a highly significant drop in the mean weight of the ventral prostate. The decrease in weight occurs more rapidly than is the case for the seminal vesicle. The weight is apparently approaching a lower limiting value of 2 to 5 mg. after 28 days of castration. This value lies between the mean weights of the ventral prostate for inhibited and regressed males in winter (Table III).

b. Histology

(i) Seminal vesicles (Plate IV, Figs. 1, 2)

The epithelium lining the seminal vesicles of a sexually active male is about 15 μ in height. After only 7 days of

castration it is reduced to about half its original height and measures about 7 μ.

By 14 days involution of the seminal vesicle epithelium is complete. It is about 4 to 5 μ high and nuclei almost fill the cells. The epithelium is similar in an animal which has been castrated for 112 days and in a sexually inactive field animal, whether regressed or inhibited.

The disappearance of secretion and involution of the muscle layers and connective tissue sheath proceeds more slowly. By 21 days there has been a reduction in the amount of secretion contained in the seminal vesicles and in the overall size of the gland. These processes are nearing completion at about 56 days.

The seminal vesicles of castrates are similar in their structure to those of regressed males in winter, having thicker muscle layers than the seminal vesicles of inhibited males.

(ii) Ventral prostates (Plate IV, Figs. 3, 4)

After 7 days of castration there is considerable loss of secretion from the follicles of the ventral prostate, but there is no noticeable change in the lining epithelium.

At 14 days there are distinct signs of involution. The epithelial height is very variable. Nuclei almost fill some of the cells. Others are still columnar, but there is an appearance of coalescence of the cell vacuoles, giving a

ragged appearance to the cytoplasm.

At 21 days the homogeneous, eosinophil secretion has almost disappeared. However, the epithelial cells are high columnar.

In an animal which has been castrated for 112 days, the ventral prostate appears in just the same state as in a sexually inactive male in winter. The folded epithelium consists of high columnar cells and a little pale staining granular secretion is present in the follicles.

(3) Discussion

Following castration of the adult rat there is complete involution of both the seminal vesicle and the ventral prostate (Moore, Hughes and Gallagher, 1930; Moore, Price and Gallagher, 1930). In rats and mice castrated before puberty, there is an anomalous maintenance of well differentiated epithelium in both the ventral prostate and the seminal vesicles. This effect is transient, disappearing in both species by the time the animals have reached 50 days of age. It is abolished by adrenalectomy (Howard, 1941).

Delost (1956b) reports the results of castrating sexually active Microtus arvalis. Involution of the seminal vesicle epithelium is complete in 8 days and minimum weight for the organ is reached after 56 days. The initial decrease in height of the epithelium of the ventral prostate also takes 8 days,

Table VIII, compiled from Delost (1956b).

Organ	State in a sexually active male	State in a castrated, immature or sexually inactive male.
Ventral prostate		
Epithelial height	c. 22 μ	c. 28 μ
Follicles	distended	epithelium folded
Secretion	abundant	small in amount.
Cauda epididymis		
Total diameter	300-550 μ	50-80 μ
Epithelial height	c. 30 μ	14-22 μ
Secretion	none	abundant in lumen and seen as granules in cells of epithelium.
Vas deferens		
Total diameter	850-920 μ	550-580 μ
Thickness of muscle layers	330-350 μ	c. 40 μ
Diameter of lumen	220-260 μ	350-345 μ
Epithelium	few folds 28-35 μ high cells with cilia	many folds 30-42 μ high cells without cilia
Secretion	present	present.

but from 15 days the epithelium increases in height again. By 28 days the epithelial height is even greater than in a sexually active adult male. This degree of epithelial development is maintained in the long-term castrate. The response is thus similar to that seen in Microtus agrestis, but events follow a rather different time course.

Other accessory organs have also been examined by Delost. The coagulating glands and the dorsal and lateral lobes of the prostate are like the seminal vesicles in undergoing atrophy. The cauda epididymis and the vas deferens, however, resemble the ventral prostate in first involuting and later showing undoubted evidence of a stimulation which is, nevertheless, quite distinct from that seen in sexually active males. This distinct type of stimulation occurs in sexually inactive winter males and in immature animals from 15 to 25 days old, as well as in castrates. Table VIII gives details of the structure of the ventral prostate, the cauda epididymis and the vas deferens in sexually active and in castrate males.

In an extensive series of experiments Delost has shown that:

1. Bilateral castration of sexually inactive males captured in winter produced no modification of the reproductive tract.
2. Bilateral castration and adrenalectomy caused the disappearance of stimulation effects in the vas deferens, cauda epididymis and ventral prostate.

3. These stimulation effects reappeared when 25 to 35 mg. of cortisone were given by subcutaneous injection over a period of 10 to 22 days, at 2 to 3 day intervals.

4. Injections of testosterone proprionate were also given to castrate adrenalectomized males. The different accessory reproductive organs showed different thresholds of sensitivity to the injected hormone. The ventral prostate was the most reactive, responding to total dosages as low as 7 to 20 µg given over a period of 6 to 17 days, at intervals of 2 days. With progressively higher doses (30 to 250 µg) stimulation of the cauda epididymis, vas deferens, seminal vesicles, lateral and dorsal prostates was achieved. A total of 7 mg. of testosterone proprionate given in 7 days, almost completely restored the accessory reproductive organs of the castrate adrenalectomized male to the degree of development they show in a sexually active male. In all cases the type of stimulation produced was that seen in a sexually active male, rather than the specific and distinct type of stimulation seen in sexually inactive, immature and castrate males. Oestradiol benzoate, progesterone and desoxycorticosterone acetate were also injected into castrate adrenalectomized males, but were unable to reproduce the type of stimulation seen in males which have only been castrated.

From these experiments Delost concludes that it is an adrenal hormone, probably a glucocorticoid, which is responsible

for the stimulation of the reproductive tract, seen in sexually inactive, immature and castrate males. The adrenal is clearly implicated. This might have been anticipated from the results of Howard (1941) with the immature rat and from the undoubted existence of an adrenal-gonad relationship (see Parkes, 1945). The dose of cortisone given was large and it is, for example, possible that it is a metabolite of cortisone rather than cortisone itself, which is responsible for the stimulation effects. Testosterone proprionate does seem to be excluded in that, even in very low doses, it does not reproduce the specific type of stimulation seen in castrates.

It has been established that in Microtus agrestis there is no evidence of stimulation of the seminal vesicles in either castrates or sexually inactive voles in winter. The ventral prostate, however, shows evidence of stimulation in winter and a similar type of stimulation develops in sexually active males when the testes have been removed. The rodent ventral prostate is generally agreed to be more sensitive to androgen than the seminal vesicle (Loraine, 1950; 1956; Delost, 1956b). The results of the experiment (p. 51), in which exogenous gonadotrophins were injected into sexually inactive Microtus agrestis, suggest that this is the case in this species also.

If the same hormone is responsible for the stimulation of the ventral prostate in both castrates and sexually inactive winter voles, it may be inferred to be of extra-gonadal origin,

and probably derived from the adrenal. It may be either

1. capable of stimulating the ventral prostate but not of stimulating the seminal vesicle; or
2. present at a sufficient level to stimulate the ventral prostate, but not the seminal vesicle.

Further experiments will be necessary to decide between these two alternatives.

C. The Effects of Exogenous Gonadotrophins on the Reproductive Tract

Voles seldom breed in mid-winter. This may be accounted for in two principal ways:

1. In the absence of suitable external stimuli, the level of secretion of gonadotrophins by the adenohypophysis is too low for gonadal activation and activity.
2. Gonadotrophins are present, but the gonads are not competent to respond to them.

There is no information about gonadotrophin levels in body fluids of the vole. Nevertheless, it was considered worth while, as a pilot experiment, to investigate the ability of exogenous gonadotrophin to stimulate the gonads of sexually inactive voles.

(1) Methods

Two readily available gonadotrophins were used: Pregnant Mare Serum gonadotrophin (PMS) (B.D.H. Serogan) and Human Chorionic Gonadotrophin (HCG) (B.D.H. Gonan). The former has follicle stimulating and luteinizing (or interstitial cell stimulating) components in the ratio of approximately two to one. The latter is almost entirely luteinizing in effect, but if used in sufficiently high concentrations, can show properties of follicle stimulating hormone (Hamburger, 1957).

a. Experiment I

17 male and 7 female animals caught in the field in January (see p. 12) were allocated at random to control and experimental groups. The 12 control animals (9 males and 3 females) received 0.2 ml. of 0.9% sterile saline daily for 4 days, given in two subcutaneous injections of 0.1 ml. B.D.H. Serogan and B.D.H. Gonan, dissolved in 0.9% saline immediately prior to injection, were given in separate injections of 0.1 ml. to the 12 experimental animals (8 males and 4 females).

Injections began on the day after capture, the animals being confined in cages kept outside, provided with food and water and covered with hay to simulate natural conditions of diffuse lighting.

Dosages	Serogan	Gonan
Day 1	1 i.u. intrap.	1 i.u. intrap.
2	2 i.u. s.c.	2 i.u. "
3	2 i.u. s.c.	2 i.u. s.c.
4	2 i.u. s.c.	2 i.u. s.c.

The increase in dosage on day 2 and subsequently was decided upon arbitrarily in the hope of getting a definite result.

All animals were killed with coal gas on the 5th day. The procedure used for fixation and weighing was as described on p. 14 f.f. Fresh weights of paired testes and fixed weights of seminal vesicles, ovaries and uteri were recorded. Mid-line sections of testes and serial sections of ovaries were prepared for histological study.

b. Experiment II

As insufficient field voles were available, the experiment was repeated using laboratory bred animals. Males only were studied, the numbers of females available being inadequate.

The males were born in the warm light conditions of the animal room. They were weaned at about 21 days and transferred to a cold room, where they were maintained at 5°C with 6 hours of light per day. Under these exaggerated winter conditions, their sexual development was inhibited. Before being used in the experiment, they were carefully examined for external signs of sexual development. All had a small penis, an unpigmented perineum and a lack of scrotal development.

Between the completion of the first experiment and the commencement of the second, Wakeling (1959) had successfully accelerated the onset of spermatogenesis in the immature rat by injections of gonadotrophins. His dose levels, reduced in proportion to the body weight ratio of voles and rats, were

used in this experiment.

Eleven inhibited males, 10 to 12 weeks old, were available. The experiment was of randomised block design with 1 missing block (Bailey, 1959). It was decided to inject hormone at two dosage levels. There were, therefore, three treatments, high hormone dosage, low hormone dosage and control, receiving no hormone. It was hoped that the gonadotrophins would induce weight changes in testes and accessory organs, as well as histological changes. Since body weight and organ weight are related, the animals were arranged in blocks, three annimals of approximately equal body weight to a block. Animals within a block were allocated at random to the three treatments.

The experimental animals were given B.D.H. Serogan and B.D.H. Gonan, by separate subcutaneous injection, in 0.05 ml. of 0.9% sterile saline. The hormones were dissolved in 0.9% saline immediately before use and injections continued daily for 7 days.

Treatment	No. of animals	Daily Hormone dosage	
		Serogan	Gonan
High	4	10 i.u.	2.5 i.u.
Low	3	5 i.u.	0.5 i.u.
Control	4	0	0

The control animals received 0.1 ml. of 0.9% sterile saline daily for 7 days, given as a subcutaneous injection.

Table XII. Effect of PMS and HCG upon ventral prostate weight in sexually inhibited male voles.

Analysis of variance

Source of variation	Sum of squares	Degrees of freedom	Mean square	Variance ratio	
Treatments	38.1250	2	19.0625	10.1450	P 0.05-0.01
Blocks	12.3267	3	4.1089	2.1868	n.s.
Residual	9.3950	5*	1.8790		
Total	59.8467	10*			

* losing a further degree of freedom because of estimation of missing plot yield.

Table XI. Effect of PMS and HCG upon seminal vesicle weight in sexually inhibited male voles.

Analysis of variance

Source of variation	Sum of squares	Degrees of freedom	Mean square	Variance ratio	
Treatments	735.9317	2	367.9659	4.3529	P 0.1-0.05
Blocks	333.7367	3	111.2456	1.3160	n.s.
Residual	422.6683	5*	84.5337		
Total	1492.3367	10*			

* losing a further degree of freedom because of estimation of missing plot yield.

Table X. Effect of PMS and HCG upon testis weight in sexually inhibited male voles.

Analysis of variance

Source of variation	Sum of square	Degrees of freedom	Mean square	Variance ratio		
Treatments	10,663.620	2	5,331.8100	3.9379	P	0.1-0.05
Blocks	11,288.757	3	3,762.9190	2.7792		n.s.
Residual	6,769.840	5*	1,353.9680			
Total	28,722.217	10*				

* losing a further degree of freedom because of estimation of missing plot yield.

Table IX. Effect of PMS and HCG upon testis, seminal vesicle and ventral prostate weight in sexually inhibited male voles.

Treatment	Number of animals	Organ weights : treatment means (mg.)		
		Testes	Seminal vesicles	Ventral prostates
High ($\bar{x}_2$)	4	94.85	20.45	5.62
Low ($\bar{x}_1$)	3	70.00	8.27	4.50
Control ($\bar{x}_c$)	4	22.70	1.52	1.37
Difference $\pm$ S.E. $\bar{x}_2-\bar{x}_c$		72.15$\pm$26.01	18.93$\pm$6.54	4.25$\pm$0.93
P		0.05-0.02	0.05-0.02	0.01-0.001
Difference $\pm$ S.E. $\bar{x}_1-\bar{x}_c$				3.13$\pm$1.05
P				0.05-0.02

The animals remained in the cold room during the injection period. They were killed with coal gas on the 8th day. The weighing and fixation of testes, seminal vesicles and ventral prostates was carried out as previously described p. 14ff. Testes were fixed in Orth (see Appendix I). The bodies were fixed in Bouin's fixative. Mid-line sections of testes were prepared for histological study.

(2) Results

a. Experiment I

No effect on testis weight, seminal vesicle weight or testis histology could be detected.

Ovulation was induced in all experimental females, as shown by the presence of new corpora lutea in their ovaries. No control female showed a corpus luteum.

There was no detectable effect on ovarian or uterine weight. Both the experimental and control groups included parous and non-parous females.

b. Experiment II

(i) Weight data

The analysis of variance for testis, seminal vesicle and ventral prostate weights, given in Tables X, XI and XII shows that there was no significant difference between blocks. For both testes and seminal vesicles, treatment differences were on the borderline of statistical significance (P = 0.1-0.05).

For ventral prostates treatment differences were statistically significant (P = 0.05-0.01).

In the analysis of variance, overall treatment differences are being considered. However, it is seen from Table IX that the mean weight of the testes of voles receiving the high dosage level of gonadotrophin was greater than that of testes of voles in the control group. The difference was statistically significant. Voles receiving the low dosage level of gonadotrophin showed an increased mean testis weight over controls, but the increase was not statistically significant.

Similarly, the difference between the mean seminal vesicle weight of the high dosage and the control voles was statistically significant; that between the low dosage and control voles was not statistically significant (Table IX).

The ventral prostates of animals receiving both the high and the low dosages of gonadotrophin showed an increase in mean weight over the controls which was statistically significant.

(ii) Histology of the testis

Controls: No control animal had sperm in its testes.

In two of the four controls the testes were identical in structure with the testes of the group of winter field animals, classed as regressed. The tubules contained only spermatogonia and Sertoli cells and the tunica was thick (Plate II, Fig. 2).

In a third control the tunica was thin. Some tubules contained primary spermatocytes, others showed only spermatogonia and Sertoli cells lining a large lumen. Mitotic activity in the spermatogonia was rare.

The fourth control had a thin irregular tunica and tubules in a variety of conditions. Some were wide and contained secondary spermatocytes. Others, especially peripherally situated tubules, showed only spermatogonia and Sertoli cells and were without a lumen.

In all control animals the interstitial cells had darkly staining, often elongate or irregularly shaped nuclei and little cytoplasm (Plate V, Fig. 1).

Experimentals: Two animals in the high and two in the low treatment group had sperm in their testes (Plate V, Fig. 3).

When contrasted with a summer field male or a sexually active laboratory male maintained in the warm light conditions of the animal room, the seminiferous epithelium appeared loosely organized. The cells were separated from each other, not packed closely together, but mitoses were quite common and the sperm were apparently normal. The tunica was thin.

Of the three remaining experimental animals, two had a thin and one a thick tunica and all showed primary spermatocytes in the tubules.

The degree of development of the interstitial cells varied,

but in all the experimental animals they were better developed than in any control. Nuclei were rounder and less darkly staining. Cytoplasm was more abundant (Plate V, Fig. 2).

(3) Discussion

There are indications from the results of both Experiment I and Experiment II that the gonads of mature, but sexually inactive animals can respond to quite low doses of exogenous gonadotrophin (van Oordt et al. 1951; van Oordt, 1956, injected total doses of up to 450 i.u. to 500 i.u. into frogs).

In Experiment I the females were, apparently induced to ovulate. In Experiment II there were increases in the weight of the testes and accessory glands, stimulation of the interstitial cells and perhaps also of spermatogenesis. It is at present impossible to know how the doses of gonadotrophin administered compare with physiological hormone levels.

The failure of the exogenous gonadotrophin to show some stimulating action on the testes and accessory glands of the males in Experiment I is not, perhaps, surprising. Both experimental and control animals were, in fact, already sexually developed, although this was not realized at the start of the experiment. In particular, the interstitial cells of the testis, which in Experiment II, show a clear cut response

to the injection of exogenous gonadotrophin, were, in the males used in Experiment I, already quite well developed.

The females used in Experiment I could not, on the basis of ovarian or uterine weight or ovarian histology, be distinguished from females in other winters. These females may not, however, have been as deep in anoestrum as is usual in mid-winter. The structure of their pituitaries (see p. 97) suggests that they may have been producing a little gonadotrophin. However, none of the control females had corpora lutea and so had not recently ovulated. All the females which had received injections of gonadotrophin had corpora lutea. The reason for absence of ovulation in controls is apparently an inadequate level of gonadotrophin, perhaps only LH, rather than the inability of the ovary to respond.

In Experiment II only the ventral prostate showed a statistically significant effect of overall treatment and statistically significant increases in mean weight at both hormone dose levels. This indicates that the ventral prostate is more sensitive to androgen than the seminal vesicle. A similar indication has been obtained by Delost (1956b) in Microtus arvalis (see also p. 50).

In considering Experiment II an explanation is required of the presence in one control animal of primary spermatocytes, in another of a few secondary spermatocytes, despite the fact that for 7 to 9 weeks these males had been maintained at 5°C

with only 6 hours of light daily. They were laboratory bred males, transferred from the animal room to the cold room at 3 weeks of age. Although not yet capable of reproduction, voles at this age have undergone a considerable amount of testicular development. Their testes weigh 50 to 100 mg. (Kennedy, 1962). The mean testis weight of control males in Experiment II was 22.7 mg. This suggests that on transference to the cold room the testes of 3 week old males undergo regression. This view is supported by the fact that the testes of two control males are identical in structure with those of sexually inactive winter males classed as regressed.

There are 3 possible ways of explaining the presence of spermatocytes in the testes of the other 2 controls.

1. Their testes are actively undergoing regression, but it has not yet been completed.
2. Activity in the seminiferous epithelium has been "frozen" at or below the level reached in the animal room, and regression will not proceed any rurther.
3. There has been continuous replacement of the seminiferous epithelium by mitotic activity of spermatogonia, but development stops short of sperm production.

The third alternative seems unlikely. A lack of mitoses and an appearance of disorganization in the seminiferous epithemium both suggest the occurrence of regression, but

whether this is proceeding actively or whether activity has been "frozen" is not known.

Comparing the stages of spermatogenesis reached in control and experimental animals it would appear that the injected gonadotrophins have been able to stimulate sperm production. However, there is considerable evidence that sperm production is a slow process and in Experiment II injections continued for only eight days. Sirlin and Edwards (1958) labelled the nuclei of spermatogonia with ^{32}P and determined the length of time taken for radio-active sperm to appear. Their results suggest that sperm production takes about 50 days in the ram and the rat, and 30 days in the mouse.

If the vole is similar to the mouse in this respect, there are two ways in which the completion of sperm production in the short time of eight days can be explained:

1. Sperm were present in the testes of the experimental animals before the injections began. The significant increases in testis weight could arise from stimulation of interstitial cells, the tubules and the earlier stages of spermatogenesis.
2. Primary spermatocytes were present in two of the control animals. They may also have been present in some of the experimental animals prior to injection. The gonadotrophins, either directly or indirectly through the stimulation of androgen production, could perhaps have induced these to complete their development. Experiments with hypophysectomized animals

show that LH is essential for spermatogenesis to occur. It probably acts indirectly, stimulating the production of androgen, which stimulates spermatogenesis. Whether FSH has any role is still uncertain (see, for example, Randolph et al., 1959).

The results of these experiments have only limited value from the point of view of the question: does the germinal epithelium change with season in its sensitivity to gonadotrophin? It is not known to what extent the doses of gonadotrophin employed may resemble hormone levels in sexually active voles.

van Oordt (1956) has presented evidence that, correlated with seasonal fluctuation in the occurrence of spermatogenesis in the frog, there is a fluctuation in the sensitivity of the germinal epithelium to gonadotrophin. Among other methods of study, he has used frogs caught at different times of year, brought to the same state of gonad atrophy by hypophysectomy and maintained in either warm or cold environments. Into these animals he has injected gonadotrophin.

Unless physiological levels of exogenous gonadotrophin can be employed, experiments similar to van Oordt's may be required to give a more convincing demonstration that changes in germinal epithelium responsiveness do not occur in voles.

Nevertheless, the results of the present experiment, taken together with the results of the investigation into the

pituitary gonadotrophin content in summer and winter voles (p. 98) suggest that seasonal variation in gonad responsiveness whether of the germinal epithelium or of the interstitial tissue is not of primary importance in the causation of vole breeding seasons.

These results may be discussed in the light of the possibility of a refractory period in mammals. The phenomenon of refractory period is well known in birds. It manifests itself in two complementary ways:

1. as an inability to respond to light stimulation for a period of time after the end of the natural breeding season;
2. as an inability to maintain gonadal activity indefinitely when exposed to stimulating light cycles in the laboratory.

It is still not certainly known at what level in the gonad-pituitary mechanism this refractoriness originates. The experiments of Riley and Witschi (1938) suggested that it was the gonad which was refractory. They injected female sparrows, both adult and juvenile with beef and horse pituitary extracts, turkey pituitary powder and PMS. In September and October only moderate increase in weight of the ovary and oviduct was produced by doses which later gave ovulation and egg shell formation. Marshall (1950) described a massive tubular steatogenesis in the testes of male birds at the end of the breeding season, together with the destruction of the old Leydig cells. It was believed that until the testis metamorphosis was complete,

the tubule lipids had disappeared and the old interstitium had been replaced by a new generation of Leydig cells, the male was unresponsive to external stimuli. No such testis metamorphosis occurs in mammals.

Clark, Leonard and Bump (1937) injected pheasant and grouse with gonadotrophic extracts in the middle of the non-breeding season. After 10 days of daily injections increases in testis weight of 119 and 500% respectively were obtained. Benoit, Mandel, Walter and Assenmacher (1950) have injected bovine adenohypophyseal extracts into ducks during the actual process of testicular regression and obtained large weight increases of 350 to 750% in 9 to 17 days.

Testis metamorphosis occurs in birds after hypophysectomy (Coombs and Marshall, 1956) or prolactin injections (Lofts and Marshall, 1956) as well as at the end of the breeding season. Lofts and Marshall (1958) showed that lipids can be dispersed from the tubules of recently hypophysectomized birds by 10 daily injections of FSH in doses as low as 0.6 i.u. Much higher doses, up to 95 i.u. resulted in sperm formation. No details are given of the source or purity of the FSH used: contamination with LH might be an important factor in producing these effects.

Such results strongly suggest that the testis changes are secondary to events elsewhere in the gonad-pituitary axis, perhaps in the pituitary itself or in the hypothalamus.

Evidence from electrical stimulation and the placing of lesions has indicated that it is via the hypothalamus that nervous and endocrine systems are integrated. The interplay between the pituitary and its target organs may also be accomplished through the hypothalamus (Harris, 1955).

In mammals there is no decisive evidence for the existence of a refractory period. Bissonnette (1938) reported that both male and female ferrets ultimately become refractory to light stimulation and that progressively greater increases in the length of light exposure per day become necessary to prolong the active phases of the cycle. This statement is apparently based on experiments with very few animals. Bissonnette (1935a) studied 3 animals on long days. Bissonnette (1935b) studied 7 animals, of which only 2 were allowed to survive for more than 2 months. He observed decrease in the size of the testes, disappearence of sperm and loss of lipid droplets from the interstitial cells in animals kept for a long time under stimulating day lengths. Since ferrets are difficult to maintain in a healthy condition in the laboratory, irrelevant factors could have contributed to the results which he obtained.

Hammond (1951) kept mink in winter on a stimulating cycle of artificial light. Vaginal smears of the treated mink went through an oestrus phase. They were returned to daylight at the end of February. At the height of the natural breeding season in mid-March the females which had already been

sexually active, all showed anoestrus vaginal smears. The males had testes in the shrunken mid-summer condition. There is no indication of the numbers of animals studied.

By contrast, if Microtus agrestis is maintained in the laboratory at a temperature of 18-21°C and with a constant daily schedule of 16 hours light per day, it will breed continuously from the time it reaches sexual maturity until it dies. Summer animals brought into the laboratory from the field, similarly continue in an active reproductive state.

It would thus seem that refractoriness neither at the level of the gonads nor in any part of the gonad-pituitary mechanism can be responsible for the change from a sexually active to a sexually inactive condition in the vole. It seems rather that the gonad changes at the end of breeding seasons are secondary to changes in the pituitary and that these are dependent upon a change in the conditions of the external environment.

CHAPTER II. THE THYROID

Delost (1951) studied the thyroid of Microtus arvalis. Animals were collected in the field in each month of the year. On the basis of histological criteria, there were considered to be two periods, one of feeble thyroid activity, from October to February, and one of much greater thyroid activity, from the end of February to the beginning of October. The greatest thyroid activity was seen in three pregnant females. These conclusions are based on very few animals in each month: 15 males and 12 females were examined in all.

In the laboratory it has been shown, in such species as rats and guinea pigs, both by histological and radio-iodine methods, that thyroid activity increases with decrease in temperature (Kenyon, 1933; Stevens et al. 1955; Brown-Grant, 1956a).

In species like Microtus arvalis and Microtus agrestis, which are active throughout the year and which do not hibernate, it might, therefore, be expected that there will be greater thyroid activity in the winter. The higher winter output of thyroxine would give a higher metabolic rate to release energy for maintenance of body temperature.

The results of Delost (1951), however, indicate that in Microtus arvalis thyroid activity is greatest during the breeding season. There are suggestions of a functional

relationship between the thyroid and the gonads (Maqsood, 1952). However, the results of Brown-Grant (1956b) show that, in the rabbit and the rat, there is no marked effect of coitus, ovariectomy, castration or stage of the oestrus cycle on thyroid activity, as measured by radio-iodine methods. In fact it seems that the role of thyroid hormone in reproduction is a permissive one, creating a metabolic environment in which gonadotrophic hormones of the pituitary and the steroid hormones involved in reproduction, can produce their effects. There are indications of increased thyroid activity in pregnancy, but not of any effect on the thyroid of gonadal activity as such.

Griesbach, Hornabrook and Purves (1956) have shown that the pituitaries of rats maintained for 14 days at 5°C show an increase in the numbers of thyrotrophic basophils, the pituitary cells which they have shown to be responsible for the production of thyrotrophic hormone (Purves and Griesbach, 1946, 1951a,b,c, 1957b). Thyrotrophic hormone and adrenocorticotrophic hormone, acting via the thyroid and the adrenal cortex, respectively, were shown to be the only factors responsible for maintenance of body temperature in these animals.

The thyroids of voles obtained from the field in mid-summer and mid-winter have been examined by weighing and by a limited histological examination. This study has been made both in the light of the rather unexpected conclusions reached

by Delost (1951) and as a background to pituitary studies. If there is a marked seasonal change in thyroid activity, the results of Griesbach, Hornabrook and Purves (1956) indicate that it may be possible to detect changes in the thyrotrophin producing cells of the pituitary, on whose secretion the thyroid changes will be presumably dependent.

(1) Weight Data for Field Animals

a. Males

The mean weight of the thyroid is higher in summer males than in winter males. The difference is statistically highly significant. Table III. However, the summer males also have the higher mean body weight. It cannot, therefore, be immediately assumed that the larger summer thyroids are a manifestation of greater relative thyroid activity. Allowance must be made for the body weight difference.

It has been a common procedure to attempt to solve this type of problem by expressing organ weight as a percentage of body weight. This, however, assumes a relationship between, in this case, thyroid weight and body weight for which there is no evidence. It has been considered preferable to attempt to determine what sort of relationship exists between thyroid weight and body weight and whether this relationship varies with season.

The regression of thyroid weight upon body weight has

Table XIII. Regression of thyroid weight upon body weight.

Probability that b differs from zero given by:

*** $P < 0.001$

	Number of animals	a	b	
Males				
Summer	25	1.4134	0.0285 ± 0.0299	n.s.
Winter	36	-0.0824	0.0844 ± 0.0145***	
Females				
Winter				
Non-parous	11	1.0444	0.0049 ± 0.0431	n.s.
Parous	12	0.9653	0.0514 ± 0.0391	
Overall	23	-0.4379	0.1062 ± 0.0274***	

been calculated for summer and winter males (Mather, 1951). The values of a and b, the constants of the equation:

$y = a + bx$ where y = thyroid weight

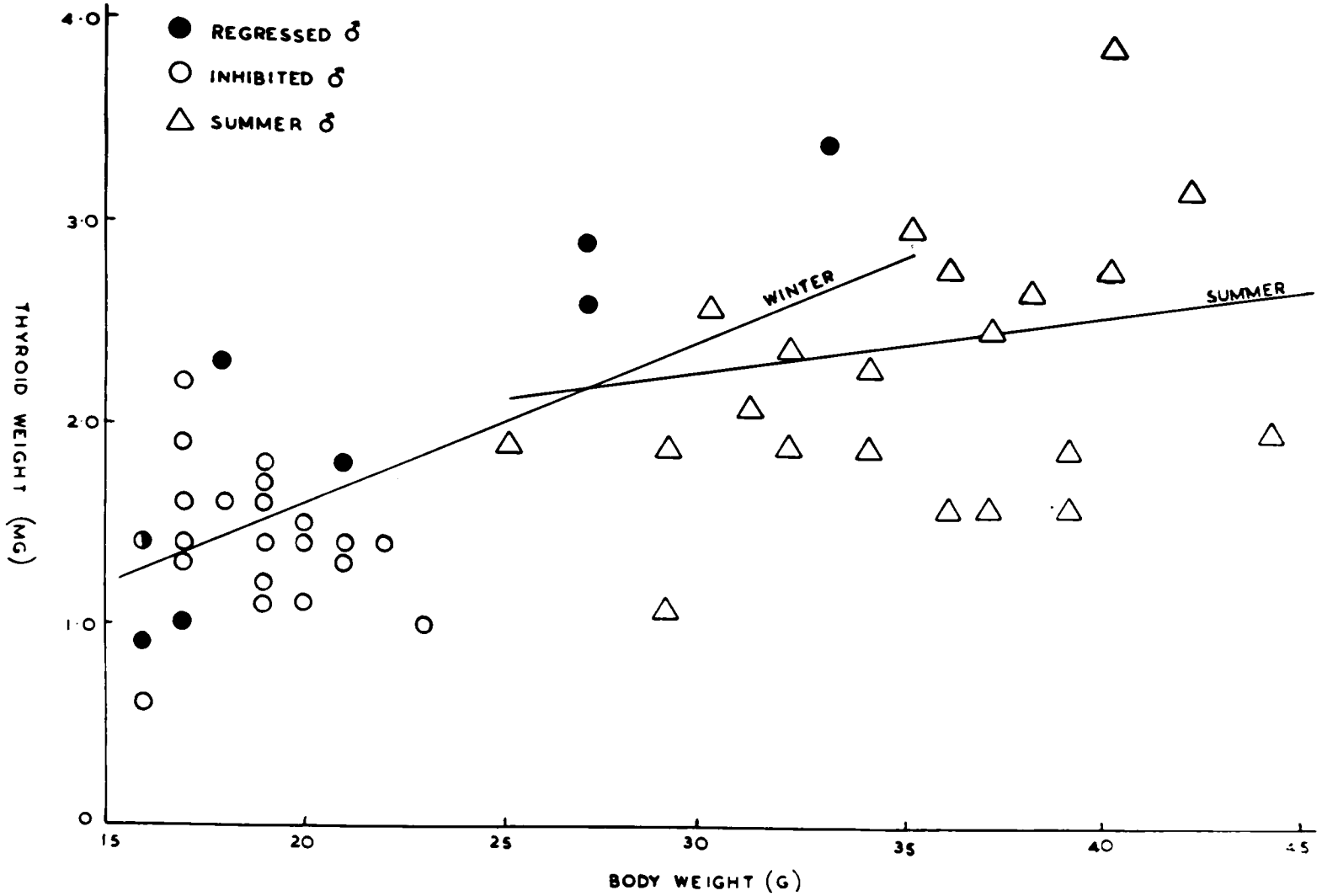
x = body weight

are given in Table XIII. (See also Text-Fig. 1). The value of b for winter thyroids is significantly different from zero, but b for summer thyroids has so large a standard error, that it cannot be shown to differ significantly from zero.

The difference between the summer and winter values of b is not statistically significant.

The difference in thyroid weight between regressed and inhibited males in winter is on the borderline of statistical significance (Table III). Plotting thyroid weight of regressed and inhibited males against their body weight suggests that any difference in thyroid weight between the two groups is a reflection of body weight differences (Text-Fig. 1).

There is a statistically significant difference in the mean thyroid weights of males in the summers of 1959 and 1960 (Table IV). Thyroids were heavier in 1959. Males in the two years also show a difference in mean body weight and, although this is not statistically significant, it is the males of the summer of 1959 which were heavier.



Text - Figure 1. The relationship between thyroid weight and body weight in male voles obtained from the field in winter and summer: observed data and calculated regression lines.

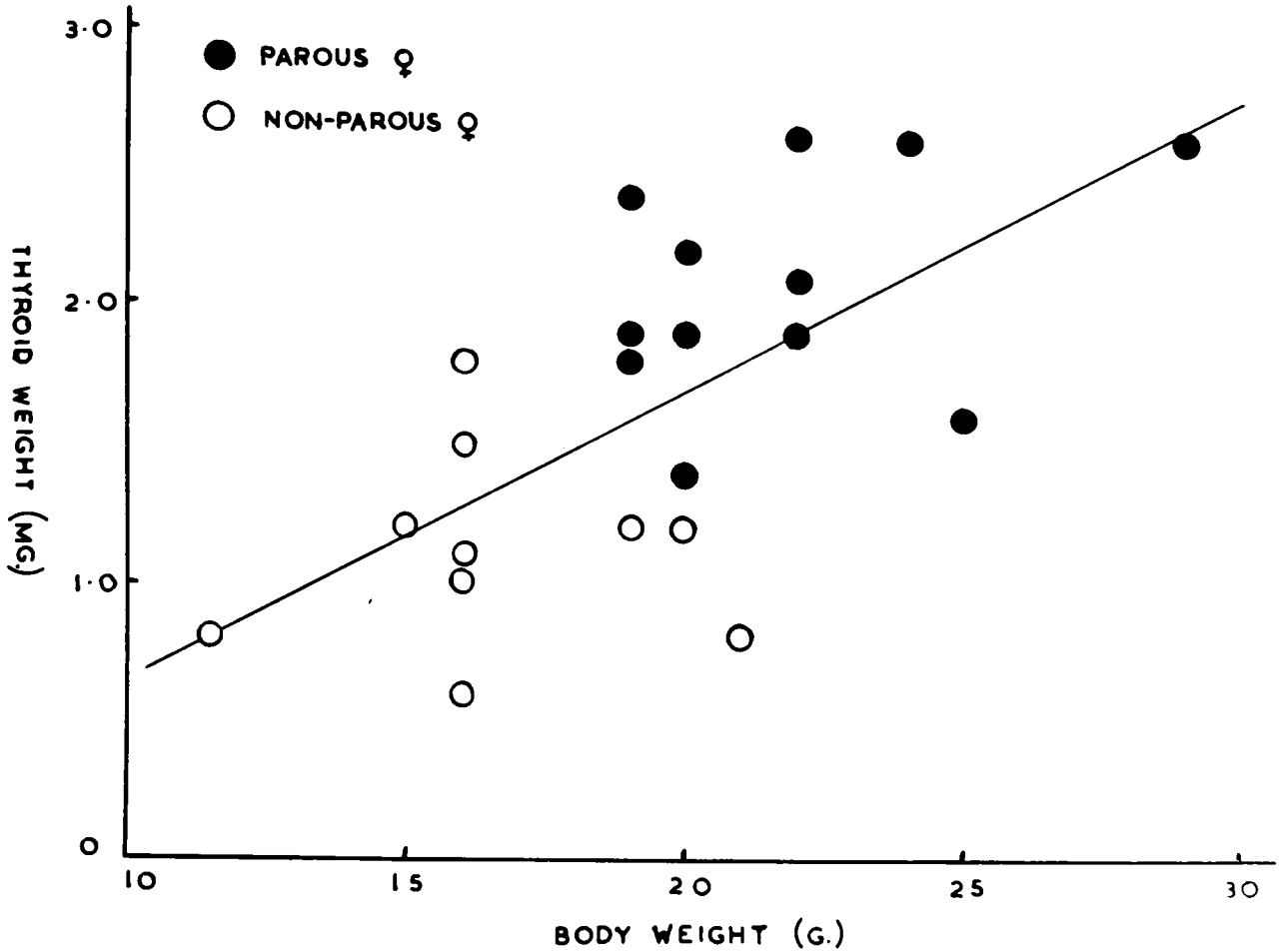
b. Females

There is no difference in the mean weights of thyroids from summer and winter females (Table V).

There is no statistically significant difference in mean thyroid weight between primipara and multipara in summer, although primipara, which have a significantly lower mean body weight, do also have the lower mean thyroid weight (Table V).

The body weight of summer females includes the weight of foetuses, so no regression analyses of thyroid upon body weight has been performed for summer females for comparison with non-pregnant winter females.

The difference in thyroid weight between parous and non-parous females in winter is highly significant (Table V). Although there is no significant difference in their body weights, it is the parous females, whose thyroids are the heavier, which are the larger animals. As with regressed and inhibited males in winter, plotting thyroid weight against body weight for parous and non-parous winter females suggests that the thyroid weight difference is dependent on body weight differences (see Text-Fig.2). Finally, the regression of thyroid weight upon body weight has been calculated for parous and non-parous females in winter (Table XIII). The slope of neither regression line differs significantly from zero, each estimate of b being accompanied by a large standard error. Nor is the difference in slope between the two regression lines significant.



Text - Figure 2. The relationship between thyroid weight and body weight in female voles obtained from the field in winter: observed data and calculated overall regression line.

If the data on parous and non-parous females is considered together, a regression line is obtained whose slope differs significantly from zero. (Text-Fig. 2).

c. Males and Females (Tables III and V)

There is no statistically significant difference in thyroid weight between males and females in winter. In summer males have a significantly higher mean thyroid weight, but this may be a reflection of their considerably greater body weight.

(2) Histology

The thyroid has been examined in only a small series of animals (see Table II). There are, however, indications of a tendency to greater thyroid activity in the summer.

In all the animals examined, the histology of the thyroid suggests some degree of activity. The epithelium lining the follicles is more or less cuboidal and shows vesicular, pale staining nuclei. There are, however, a number of variable factors:

1. The height of the epithelium.
2. The amount of colloid in the follicles.
3. The numbers of vacuoles around the edge of the colloid mass.

Histological criteria probably do not give a very accurate indication of thyroid activity. However, in general, an

inactive thyroid has follicles lined by a low epithelium. A large mass of colloid, which is not vacuolated at its edges, is contained within each follicle. An active thryoid has follicles lined by a low columnar epithelium, which may be folded. There is little colloid and many vacuoles occur around the edges of the colloid mass. The significance of these vacuoles is not known precisely. They are probably an artifact, produced in fixation. They are, nevertheless, useful in determining the state of activity of the gland. They probably indicate a changed consistency of the colloid, associated with resorption (see Bailey, 1958).

In eight of eleven summer animals and four of nine winter animals examined, the epithelium lining the follicles is cuboidal. The colloid is excavated by a considerable number of vacuoles, but remains abundant (Plate VI, Fig. 1). The eight summer animals include five females in various stages of pregnancy and three males. Of the four winter animals, three are males and one a non-parous female.

The other three summer animals examined, two males and a female which was not obviously pregnant, have very little colloid in the follicles, extensive vacuolation of what colloid is present and a follicular epithelium which is low columnar (Plate VI, Fig. 2). No winter animal has a thyroid which, histologically, appears so active.

On the other hand, there are five winter animals in which the colloid is abundant and there are only a few small vacuoles. From three females, two parous and one non-parous, they are virtually absent. In two males the vacuoles are few (PlateVI, Fig. 3).

A summary of these results is given in Table XIV.

Table XIV

Level of Thyroid activity as judged by histological criteria	Numbers of animals showing level of activity specified	
	Winter	Summer
Inactive	5	0
Moderately active	4	8
Active	0	3

(3) Discussion

Since the summer and winter values of b, the regression coefficient relating thyroid to body weight in male voles, do not differ significantly from each other, there is no convincing evidence that the relationship of thyroid to body weight changes with season. The possibility cannot be excluded that the difference in thyroid weight between male voles in summer and in winter is due simply to their difference in body weight. Had there been a significant difference between the regression coefficients, a difficulty of interpretation would still have

arisen. The distributions of body weights for summer and winter males only just overlap. Winter animals might then simply be lighter or younger animals occupying a different position in a general growth curve relating thyroid to body weight, independent of season.

In all the other comparisons which have been made, there are suggestions that differences in mean thyroid weight are explicable in terms of body weight differences.

The limited observations made on the histology of the thyroid indicate a tendency to greater thyroid activity in the summer and this accords with the evidence about seasonal variation in thyroid function which is available. Delost (1951) found that the thyroid of Microtus arvalis was more active in the breeding season. Eickhoff (1952) found that the thyroids of wild rabbits and hares shot in winter were histologically less active than those of animals shot in summer and autumn. Kracht (1954) confirmed this, observing that in wild rabbits shot in winter many thyroids are inactive, whereas in late summer and autumn active thyroids occur more frequently.

Zalesky (1935) studied the thyroid of the ground squirrel, Citellus tridecemlineatus. This species hibernates in winter and in hibernating animals, with their low metabolic rate, the thyroid appeared very inactive. An increase in thyroidal activity occurred after emergence from hibernation and reached

a peak in April or May. This activity was not correlated with sexual activity in the male, but pregnant and, to a lesser extent, lactating females showed particular hypertrophy and hyperplasia. Delost (1951) noted particular thyroid activity in pregnant Microtus arvalis.

This association of pregnancy and marked thyroid activity has not been seen in Microtus agrestis. The summer females, whose thyroids were examined, include animals in early, mid and late pregnancy. The only female with a thyroid considered, on the basis of histological criteria, to be very active was not obviously pregnant, though it may have been in the first week of pregnancy.

Much more extensive histological studies and probably the use of radio-iodine techniques, will be necessary to determine the nature and extent of seasonal changes in thyroid activity in Microtus agrestis. It is clear that, if there is a seasonal change in thyroid activity, it is slight compared with the change in reproductive activity. It may be anticipated that the cells of the pituitary which are responsible for the production of thyrotrophic hormone will not show striking changes.

CHAPTER III. THE ADENOHYPOPHYSIS

Because of the trophic control which the adenohypophysis exercises over the other endocrine organs, it may be expected to be important in bringing about any seasonal endocrine change and, therefore, to show seasonal changes in its own histology, cytology and hormone content.

The laboratory experiments of Clarke (1957) have indicated the type of pituitary cytological differences which may be expected between mature breeding and non-breeding animals.

The study of the pituitaries of voles obtained from the field has been accompanied by laboratory experiments, in which the function of two types of pituitary cell has been investigated by means of castration and the administration of goitrogens. These methods have been used to identify the gonadotrophin and thyrotrophin producing cells of the rat's pituitary (Griesbach and Purves, 1945; Purves and Griesbach, 1946, 1951b, 1955, 1956).

A. Description of cell types in the adenohypophysis of normal, sexually active male voles.

The pituitaries were fixed, dissected out, embedded and sectioned as previously described (see p. 14).

They were stained by the methods given in Appendix II.

This description applies to the adenohypophyses of males obtained from the field in summer and to laboratory bred males.

Five cell types have been recognized:

1. Round or oval PAS positive cells (Plate VII , Fig. 1).

These contain granules which react strongly and are magenta after application of the McManus periodic acid-Schiff (PAS) technique. The granules are aldehyde fuchsin (AF) negative and stain blue with Crossmon. They are quite large and may be spread evenly throughout the cytoplasm or may show varying degrees of aggregation. In some cells the aggregated granules appear as a band around the nucleus, separated from it and the cell membrane by an unstained space. The nucleus of these cells is round. A negative image of the Golgi body is rarely seen. The diameter of these cells is approximately 8 to 11μ and they comprise about 9% of the cells of the adenohypophysis. They occur throughout the adenohypophysis. They are seen in particular concentrations in the lateral areas of the gland, in the angle adjacent to the intermediate lobe and in the region near the pituitary stalk. In this last region they may assume various vacuolated forms. They are frequently found in close relation to capillaries.

2. Angular PAS and AF positive cells (Plate VII , Fig. 2)

These contain granules which react strongly with both PAS and AF. With the former the granules are magenta, with the latter purple. In a Crossmon preparation the granules are blue. These cells vary in shape and in the abundance of granules. They usually appear as distinctly angular, but may

be barely so. Clearly the shape assumed by an angular cell in a given section will depend on how the cell has been cut. They may partially surround other cells, usually acidophils. They frequently show long, thin processes extending between neighbouring cells. The nucleus is round. A small negative image of the Golgi body is frequently a feature of these cells. Because of irregularity in shape it is difficult to give an indication of the size of angular cells. The maximum diameter of the main part of the cells is usually about 13 μ. At right angles to this it may be much thinner, about 7 μ. The thin processes may extend a considerable distance, 10 to 12 μ, beyond the main part of the cell. About 6% of the cells of the adenohypophysis are angular cells. They occur throughout the gland. In particular they are found in the mid-line. They extend laterally, especially in the posterior third of the gland. They are frequently mainly dorsal in their distribution extending evenly throughout the thickness of the tissue only in the region posterior to the neural lobe.

3. Round yellow cells (Plate VII , Fig. 3)

They have a round nucleus and rather narrow rim of cytoplasm. Distinct granules are not usually visible and the cytoplasm appears to stain homogeneously yellow with orange G and orange with Crossmon. These cells show no reaction with PAS or AF. They are about 7 to 8 μ in diameter and account for about 45% of the cells of the adenohypophysis. They are

distributed throughout the gland.

4. Red Cells (PlateVII, Fig. 3)

These cells have a more or less oval nucleus and little cytoplasm. Occasionally they are larger, rather irregular in shape and contain a large negative image of the Golgi body. Because of their small size and sparse granulation, the staining properties of these cells are difficult to define. In PAS treated sections, counterstained with orange G, they are a different colour from the round yellow cells. They appear to take up both PAS and orange G and are a pink shade. In Crossmon they are stained with acid fuchsin and appear red. They are about 6 to 7 μ in diameter, account for about 40% of the adenohypophyseal cells and occur throughout the gland.

5. Large oval cells (PlateVII, Fig. 4)

These are the largest cells in the adenohypophysis, reaching a maximum diameter of about 17μ. They are few in number, forming about 1-2% of the adenohypophyseal cells. They are oval, with a round or oval, often very large nucleus. They are finely granular. The granulation may be spread evenly throughout the cell or may tend to form strands, radiating from the nucleus. The granules are weakly PAS and AF positive. They do not stain with orange G. In Crossmon preparations they are a pale grey-blue. These cells often contain a single large granule, weakly PAS and AF positive, staining blue with Crossmon and homogeneous in

appearance.

Cells which are totally devoid of specific granulation and which may be considered as chromophobes are very rare.

B. Analysis of cell function

(1) Experimental investigation: the effects of castration on the adenohypophysis

a. Methods

The technique of castration has been described (p. 42).

Pituitaries were fixed in formal sublimate, dissected out and prepared for histological examination as previously described (p. 14). Details of staining methods are given in Appendix II.

b. Results

Striking changes take place only in the round or oval PAS positive cells. These cells are abundant and well granulated in all control animals.

After 7 days of castration the only strongly PAS positive cells in the adenohypophysis are angular. Empty round or oval cells, often lying along the capillaries, are, because of their shape and position, considered to be degranulated round or oval PAS positive cells (PlateVIII, Fig. 1).

At 14 days the PAS reaction of these cells is still very slight. Some of the cells appear vesiculated with strands of PAS positive material crossing the unstained space between the

nucleus and the periphery of the cell (PlateVIII, Fig. 1).

By 21 to 28 days the reaction to PAS has intensified. Cells have appeared which contain one to many colloid filled vesicles. The colloid stains pale pink with PAS and pale blue with Crossmon. It may, itself, contain round unstained vacuoles (PlateVIII, Fig. 2). The formation of colloid filled castration cells has long been recognized as a specific response to castration in the rat (see Purves and Griesbach, 1955).

At 56 days the adenohypophysis contains only such castration cells, together with the other four cell types described in the normal male, which are, apparently, unchanged. The castration cells are distributed throughout the adenohypophysis in a manner similar to the round or oval PAS positive cells of the normal animal.

In animals castrated for 84 and 112 days, the adenohypophysis contains typical castration cells, but also rather small, round or oval, well granulated cells. The granules are intensely PAS positive. These cells have a prominent negative image of the Golgi body (PlateVIII, Fig. 3).

c. Discussion

Following castration the round or oval PAS positive cells of the vole's pituitary degranulate and are apparently transformed into colloid filled castration cells, though no intermediate stages in this transformation have been detected.

The other four cell types of the adenohypophysis are, apparently, unchanged. Together with their reaction to PAS, this suggests that the round or oval cells secrete gonadotrophin, that is that they are gonadotrophs, following the terminology of Purves and Griesbach (1951a).

This reaction of round PAS positive cells is similar to that which has been described in the rat. In the rat, Purves and Griesbach (1952, 1954, 1955) have been able to identify two types of gonadotrophs, peripheral, FSH producing cells and central LH producing cells. In the vole it has not, so far, been found possible to distinguish FSH and LH producing cells.

The occurrence of rather small round or oval cells with intensely PAS positive granulation in the pituitaries of voles castrated for 94 and 112 days suggests that regranulation of gonadotrophs may take place in long term castrates. It is not, however, known whether the well granulated cells arise by transformation of colloid filled castration cells or from some other source. Regranulation of FSH producing cells has been noted at 336 days in the rat. (Purves and Griesbach, 1955).

The structure of castration cells appears to be more variable in the vole than in the rat. The typical castration cell of the rat is the "signet ring cell" with a single colloid vesicle. The vole shows in addition more bizarre forms with multiple colloid vesicles.

The round or oval PAS positive cells which occur in the region of the pituitary stalk undergo degranulation and accumulation of colloid in response to castration. They are, therefore, presumably gonadotrophic in function. Why they should so frequently assume vesiculated forms in summer animals is not known.

(2) Experimental investigation: The effects on the adenohypophysis of the administration of methylthiouracil

a. Methods

Thiourea, as a 0.25% solution in drinking water, was used by Griesbach and Purves (1945) and Purves and Griesbach (1946) as a goitrogen in rats. At this dilution, thiourea was found to be highly toxic to voles.

0.04% methylthiouracil, however, was not toxic and produced appreciable enlargement of the thyroid.

It was administered in the drinking water. The water bottles were emptied out and refilled with a freshly prepared solution twice weekly.

25 litter mate pairs of males have been studied. From each litter mate pair, one animal was chosen at random to receive the goitrogen.

Both control and experimental animals were maintained, one animal to a cage, at 18 to 21°C and with 16 hours of light daily. They had an unlimited food supply and were weighed

Table XV. Effects of methylthiouracil administration: male organ weights

	Number of animals	Mean weights of specified organs		Mean difference ± S.E.	P
		Control	Treated		
Thyroid (mg.)	25	2.08	6.57	+4.49 ± 0.78	< 0.001
Initial body weight (g.)	26	28.58	27.46	-1.12 ± 0.86	n.s.
Final body weight (g.)	26	30.15	28.38	-1.77 ± 0.85	0.05-0.02
Change in weight during experiment (g.)	26	1.57	0.92	-0.65 ± 0.65	n.s.
Testes (mg.)	26	344.34	332.21	-12.13 ± 19.45	n.s.
Seminal vesicles (mg.)	26	116.84	120.37	+3.53 ± 8.46	n.s.
Ventral prostates (mg.)	26	32.56	31.77	-0.79 ± 3.85	n.s.
Adrenal (mg.)	26	4.45	4.53	+0.08 ± 0.17	n.s.

once weekly. They remained in good health throughout the experiment.

The animals were killed 2, 4, 7, 14, 28 and 56 days after the beginning of the experiment. Fresh weights of adrenals and testes were recorded. The pituitary was fixed in formal sublimate and the body in Bouin's fixative. Fixed weights of the thyroid, seminal vesicles and ventral prostates were obtained as previously described (p. 14).

Pituitaries and thyroids were prepared for histological and cytological examination (see p. 15 and Appendix II).

b. Results

(i) Weight data

The mean weight of the thyroid in animals receiving goitrogen is considerably greater than the mean weight of the thyroid in controls. The difference is statistically highly significant. (Table XV).

Thyroid enlargement increased with increase in the length of treatment. The slope of the regression line relating thyroid enlargement to length of treatment was significantly different from zero (Table XVI).

The mean weight of the adrenals, the testes, seminal vesicles and ventral prostates show no significant differences between the control and experimental groups (Table XV).

The difference in the mean initial body weights of control and experimental animals is not statistically significant.

Table XVI. Regression of increase in thyroid weight (y) upon days of treatment with methylthiouracil (x) in male voles.

$$y = 1.7165 + 0.1091 \; x$$

$$b = 0.1091 \pm 0.0275 \qquad P < 0.001$$

The difference in their final body weight is statistically significant, the mean body weight being lower in the experimental group. There is no significant difference between the mean change in weight during the experiment of control and experimental animals (Table XV).

(ii) Histology

Thyroid: Changes in thyroid histology could be detected even after only two days of methylthiouracil administration.

In control animals the thyroid epithelium varies from low cuboidal to cuboidal. The nuclei of the epithelial cells tend to be slightly irregular in outline. Follicles contain abundant colloid.

In animals treated with goitrogen for two days the height of the epithelium was increased and is cuboidal to low columnar. The nuclei are all vesicular. Staining of the follicular colloid is faint and it is extensively vacuolated.

The thyroids of animals which have received goitrogen for longer periods show further increase in epithelial height. The colloid stains much more weakly than in the controls, it becomes increasingly vacuolated and sometimes seems to have been lost completely.

Pituitary: Marked changes take place only in the angular cells containing PAS and AF positive granules. Such cells are abundant in the adenohypophyses of all control animals.

The adenohypophyses of voles treated with goitrogen for 28 and 56 days possess numerous, greatly enlarged, colloid filled cells (Plate IX , Fig. 2). The colloid often contains unstained vacuoles and is, itself, faintly PAS and AF positive. In a Crossmon preparation it stains blue. These cells resemble the thyroidectomy cells described first in the rat by Kojima (1917).

In animals which had received goitrogen for only 2 days, the angular cells are apparently more intensely stained with AF than are the angular cells of controls. There are also present some angular cells which, while retaining typical AF positive granulation, also contain patches of colloid, resembling that seen in thyroidectomy cells. This colloid sometimes contains unstained vacuoles (Plate IX , Fig. 1).

Such cells, with both colloid and AF positive granules, are also present in animals which had received goitrogen for 4, 7 and 14 days. There are also an increasing number of thyroidectomy cells.

Colloid-containing thyroidectomy cells are found throughout the adenohypophyses of voles treated with goitrogen for 28 and 56 days. In distribution they are similar to the angular AF positive cells of the normal pituitary. In such animals the other four cell types described for the normal male are present and apparently unchanged.

The structure of thyroidectomy cells differs from that of castration cells. They are larger, irregular in shape and contain massive amounts of colloid which usually contains unstained vacuoles. Castration cells are smaller, roughly round or oval and the colloid does not so frequently contain unstained vacuoles.

c. Discussion

The function of the angular PAS and AF positive cells of the vole's pituitary, and their identity as an independent cell type, has been suggested by the transformation which they undergo in animals treated with methylthiouracil.

They are similar in their morphology and staining properties to the thyrotrophs of the rat, described by Purves and Griesbach (1951b,c). Like rat thyrotrophs they react specifically to thyroxine deficiency, but the nature of the reaction appears to be somewhat different. This may depend on use of a different goitrogen (methylthiouracil, instead of thiourea or iodothiouracil), as well as on possible species differences in the reactions of the thyroid-pituitary axis.

The establishment of thyroidectomy cells in the rat, following surgical thyroidectomy or the administration of goitrogens, takes 7 to 14 days and is preceded by total degranulation (Purves and Griesbach, 1956). In voles, thyroidectomy cells may appear as early as two days and there is no initial degranulation.

The initial total degranulation of rat thyrotrophs makes it difficult to be sure that thyroidectomy cells do arise from them. In order to establish this identity, Purves and Griesbach (1956) used general morphological criteria. They also described T granules, intensely PAS positive bodies, which are characteristic of fully developed thyroidectomy cells in the rat. They are larger than the normal granules of thyrotrophs and differ from them in their solubility and reaction to AF. In rats given low doses of goitrogen, they saw undoubted thyrotrophs which, nevertheless, contained T granules.

T granules have not been seen in the vole. However, degranulation does not precede hyalinization, so that the evidence of identity of thyroidectomy cells with thyrotrophs, for which Purves and Griesbach (1956) looked unsuccessfully in the rat, has been provided in the vole. In animals which have been receiving goitrogen for short periods, cells are present which contain both colloid and specific AF positive granulation.

The change in pituitary cytology which occurs in animals receiving methylthiouracil has been connected with the effect which the goitrogen has in inhibiting thyroxine secretion. There is no significent difference in the mean weights of gonads, adrenals or accessory organs between control and experimental animals. There is, however, a suggestion of

retardation of general body growth in the experimental animals. Their mean final body weight is lower than that of controls and the difference is statistically significant.

However, the mean initial body weight of the experimental animals was, by chance lower than that of the controls, though the difference is not statistically significant.

The fact that during the experiment, the treated animals did increase in weight and that their mean increase in weight, though less than that shown by controls, is not significantly different from it, indicates that any effect of treatment on general body growth was not marked.

It is presumably because of the slight initial weight difference and the slight, perhaps chance differences in growth that the difference in the final body weights of control and experimental animals reaches statistical significance.

(3) The adenohypophysis of female field voles in summer

All the females obtained from the field in summer were either pregnant or lactating or were both pregnant and lactating.

Their adenohypophyses differ from those of sexually active males in three respects:

1. The gonadotrophs show considerable variation in density of granulation and in the intensity of the reaction of their granules with PAS. They may be partially degranulated, only

weakly PAS positive and, therefore, rather inconspicuous.

2. The round yellow cells appear to be reduced in numbers.

3. There is a striking development of the red cells. In a male these are, in the main, small and sparsely granular (Plate VII, Fig. 3). In a pregnant or lactating female they have a more or less oval nucleus and a very large and prominent negative image of the Golgi body. In shape they are rather irregular, often roughly rectangular. In size they are about 6 by 9 μ. They contain a few very small PAS positive granules and also take up orange G, to appear pink in PAS-orange G preparations. In Crossmon they take up acid fuchsin and are red. In most virgin laboratory females these cells are no more prominent than in a sexually active male. Some virgin laboratory females, housed alone or in pairs, appear to pass into a pseudopregnant state. This is characterized by possession of enlarged and prominent mammary glands. The cause of this phenomenon is not known, but it may be induced by the activity or odour of males in adjacent cages (cp. Whitten, 1958), In such females the red cells resemble those in pregnant females, in having a large negative image of the Golgi body and quite intense staining properties (Plate IX, Fig, 3).

(4) General discussion of functional differentiation of the cells of the vole's pituitary

Purves and Griesbach (1957a), in studying the dog pituitary, subdivided the chromophil cells into acidophils and basophils. They defined "basophils" in terms of their content of cytoplasmic granules composed of soluble glycoprotein. The granules, therefore, stained strongly with PAS after ordinary fixation, but they could be removed by extraction in cold phosphate buffer of pH 7.5 before fixation.

In terms of this definition it seems that the oval and angular PAS positive cells of the vole's pituitary, that is the gonadotrophs and thyrotrophs, should be classed as "basophils"; that the round yellow cells and probably also the red cells should be classed as "acidophils". The status of the fifth cell type in this classification is not established.

The terms "acidophil" and "basophil" are retained for convenience of reference. It is, however, recognized that how a particular cell stains will depend on the conditions of staining, quite apart from the fact that in trichrome methods, which satisfactorily distinguish "acidophils" from "basophils", no basic stain is used. These categories are, in fact, rather artificial and perhaps of little significance. Their use does not imply the acceptance of any particular theory of cell relationship.

Castration and the administration of goitrogen has enabled the function of two of the cell types in the vole's pituitary

to be investigated. The existence of gonadotrophs and thyrotrophs as distinct cell types is suggested by their differences in morphology, staining properties and their reaction to castration and chemical thyroidectomy, resulting in the formation of two different types of colloid filled cells.

Of the other three cell types which have been recognized, a function can be ascribed only to the red cells. These cells are conspicuous in pregnant females and in females showing signs of mammary development. In such animals red cells have a large negative image of the Golgi body and considerable amounts of granulation. This suggests that they may be the source of prolactin.

In several species of mammal two types of acidophil have been identified. One type stains with orange G. The other stains with azocarmine, acid fuchsin and erythrosin. The species studied include the dog (Wolfe, Cleveland and Campbell, 1932; Goldberg and Chaikoff, 1952; Purves and Griesbach, 1957a), the cat (Dawson, 1946), the rabbit (Friedgood and Dawson, 1938), the bat (Herlant, 1956), the monkey (Dawson, 1954a), the ferret (Holmes, 1951, 1960), the sheep (Clarke and Purves, 1960). The cells which stain with azocarmine, acid fuchsin and erythrosin show fluctuations in activity in relation to ovulation, pregnancy, lactation, and are considered to be the source of prolactin. In at least three species: the dog (Purves and Griesbach, 1957a), the ferret (Holmes, 1959, 1960) and the sheep (Clarke and Purves, 1961; Clarke,

1961) these cells, as in the vole, have been shown to react weakly with PAS.

In the rat difficulty has been experienced in differentiating two types of acidophil by staining methods. Lacour (1950) has used the kresazan-orange stain of Romeis successfully. Dawson (1954b) reports sporadic success using a modification of the azan method. Purves and Griesbach (1952) were unable to achieve differentiation of acidophils by staining methods. However, two types of acidophil could be detected by their response to experimental treatment. One type, situated in the interior of cell cords, degranulated in animals which were castrated or treated with oestrogen. The second type was more prominent in these conditions, but degranulated in thyroxine deficiency, when the first type persisted. They were suggested as the sources of prolactin and growth hormone, respectively.

In the vole no reaction to castration has been detected in the red cells, considered to produce prolactin. They do, however, show the type of staining reaction seen in what are probably the prolactin producing cells of other mammals.

It is possible that the round yellow acidophils are the source of somatotrophin. They are not degranulated in the animals which have received methylthiouracil and show fully developed thyroidectomy cells. However, acidophil degranulation occurs only in extreme thyroxine deficiency. Griesbach and Purves (1945) and Purves and Griesbach (1946) used thiourea to

produce a state of thyroxine deficiency in which basophil changes occurred, but the acidophils remained fully granulated. It is possible that voles receiving 0.04% methylthiouracil are still producing enough thyroxine to maintain their somatotrophin producing acidophils.

This view is supported by the fact that the change in weight of treated and control animals, during the course of the experiment, is not significantly different.

On the other hand, there is some suggestion of a reciprocal relationship between the two types of acidophil in the vole. In pregnant and lactating females round yellow acidophils appear to be reduced in numbers. The red cells have developed a large negative image of the Golgi body and stain more intensely. They are, therefore, more conspicuous than at other times and have perhaps increased in numbers. It is possible that round yellow cells and red cells represent phases in the secretion cycle of the same cell type.

It has been noted that angular PAS and AF positive cells may partially surround other cells, usually acidophils. This is similar to the phenomenon of 'cupping' described in the dog's pituitary by Purves and Griesbach (1957a).

There is no information available about the function of the large oval cells.

C. Seasonal Change in Adenohypophyseal Function

(1) Cytological evidence

A description has already been given of the adenohypophyses of adult male and female field voles.

a. The adenohypophysis of sexually inactive winter field voles

Regressed and inhibited males, parous and non-parous females have been studied, but no differences in their pituitary cytology have been detected.

All showed marked changes in the gonadotrophs. The well granulated cells seen in summer animals are almost entirely replaced by vesiculated cells (PlateVIII, Fig. 4). The contents of the large vesicles which they contain do not stain. The strands of material which form the boundaries of these vesicles contain intensely PAS positive material. There is thus an appearance of intense PAS staining around the nucleus, at the periphery of the cell and crossing the unstained space between.

In some winter animals there may also be occasional well granulated gonadotrophs. These appear smaller than in summer animals.

No marked changes take place in any of the other cell types. The red cells are not conspicuous, as in a summer male or virgin female. In those pituitaries which show the most extreme development of vesiculated cells, it is sometimes difficult to detect thyrotrophs. However, careful study

reveals that they are pale, but well granulated.

b. The adenohypophyses of animals in the winter of 1958-59

The males caught in the winter of 1958-59 showed considerable sexual development. Their testes either weighed more than 100 mg. and contained sperm or weighed less than 100 mg. and contained spermatids or spermatocytes.

The pituitaries of these males contained gonadotrophs in a variety of conditions, but the majority of males in both groups show both vesiculated and well granulated gonadotrophs. The structure of the vesiculated gonadotrophs is as in sexually inactive males. The granulated gonadotrophs contain rather large, aggregated granules which are intensely PAS positive.

The relative proportions of vesiculated and well granulated gonadotrophs varies, but, in the majority of males with testes less than 100 mg. in weight, vesiculated gonadotrophs were less abundant than well granulated ones.

One male with sperm in its testes had a pituitary containing only vesiculated gonadotrophs.

The females of the 1958-59 winter could not be distinguished from those of other winters on the grounds of organ weight or the histology of the ovaries. However, their pituitaries show in the main the type of structure described for males, that is their pituitaries contain both vesiculated and granulated gonadotrophs. In 2 of 9 females only vesiculated gonadotrophs could be detected.

(2) Bioassay of the gonadotrophin content of the pituitaries of male field voles in summer and in winter.

The effect of injections of exogenous gonadotrophin on the gonads of mature, but sexually undeveloped voles suggests that the gonads are able to respond to gonadotrophin. Therefore, the low weight and the histology of the gonads of the winter field voles which showed vesiculated gonadotrophs in the adenohypophysis, indicates that these cells are not secreting gonadotrophin or are secreting it at very low levels. They may, however, be storing gonadotrophic hormone. It is possible that absence of sexual development could be associated with a high pituitary content of gonadotrophin. There is more than one way in which such a situation could be brought about. For example, if, during the non-breeding period secretion of gonadotrophin is markedly reduced or ceases altogether, but synthesis continues, then there would be a high pituitary content of gonadotrophic hormone, but no sexual development. Again, supposing the possibility of storage for some considerable period, if in the autumn synthesis continued, even for only a limited period after secretion had ceased, then the winter pituitary might have a high gonadotrophic potency.

If, however, both synthesis and secretion cease together in the autumn, or if cessation of secretion is caused by cessation of synthesis, then the winter pituitary should have a low gonadotrophic potency.

Similarly, in the summer pituitary the gonadotrophin content will depend on the relationship between synthesis and secretion.

The gonadotrophin content of winter and summer pituitaries has been investigated by means of bioassay.

Males only were used in this study. In females the interpretation of results would have been complicated by the probable effects of oestrus cycles and pregnancy on hormone content of the pituitary.

a. Methods

The assay method used was the mouse uterus test (Levin and Tyndale, 1937). This measures a mixture of FSH and LH activities (Loraine, 1956).

Ladman and Runner (1951) have studied various methods available for the estimation of gonadotrophins. Their source of gonadotrophin was mouse pituitaries collected from females approximately 2 hours after parturition. Taking as one of their end points the amount of unfractionated pituitary gland, in terms of pituitary equivalents, which elicited at 100% increase in the uterus to body weight ratio of immature mice, they found that the critical change occurred at between 2 and 4 mouse pituitaries. 2 pituitary equivalents produced a 77% increase in uterine weight; 4 pituitary equivalents a 296% increase.

It seemed quite likely that the pituitaries of sexually mature voles might have a potency of the same order as Ladman and Runner's mouse pituitaries. It was, therefore, decided to inject an amount of unfractionated pituitary gland equivalent to four summer vole pituitaries into each assay animal.

A trial assay, using mouse pituitaries, was first performed, to become familiar with technique. The mean uterine weight of the mice receiving the pituitary extract was greater than that of the controls, but the difference was not statistically significant. This was probably due to the fact that female pituitaries were used. Unlike the females used by Ladman and Runner (1951) these mice had not been recently pregnant and were not lactating. The greater gonadotrophic potency of the pituitary of mature male rats as compared with mature non-pregnant females, undergoing cyclic oestrus activity, has long been known (Evans and Simpson, 1929; Purves and Griesbach, 1954).

Field animals were caught by the methods previously described (p. 12).

Whole pituitaries were dissected out immediately the animals were killed. No attempt was made to separate neurohypophysis and adenohypophysis. The pituitaries were placed in a weighed tube, which was plugged with cotton wool and kept cold in a bath of alcohol at a temperature of -5 to +5°C. When all the animals had been dissected, the tube containing the pituitaries was reweighed and placed in a vacuum tube,

attached to a freeze drying apparatus. Freeze drying continued overnight, the vacuum tube being packed round with ice. The pituitaries were stored under vacuum at -16°C until assayed.

The body weight, fresh testis and adrenal weights and the Bouin fixed weight of the seminal vesicles and ventral prostates of the trapped animals were also recorded.

For assay the pituitaries were ground up and suspended in 0.9% sterile saline, to give 8.8 mg. of fresh weight pituitary tissue per 0.15 ml. This weight of tissue represents the equivalent of 4 summer pituitaries or 8 winter pituitaries and was the total amount received by each assay animal. The suspension was stored in a corked tube at -16°C between injections.

There is a large weight difference between the pituitaries of summer and winter voles. This is partly, though perhaps not wholly, accounted for by body weight differences. It is, therefore, important that equal weights of pituitary tissue, rather than equal numbers of whole pituitaries should be injected into assay animals.

The summer collection consisting of 20 animals was made on June 14th 1960 and the pituitary material was stored for 5 weeks before being assayed.

The lower weight of winter pituitaries, combined with the winter decline in numbers of voles, made it difficult to

obtain an adequate supply of winter material. A series of catches was made from December 20th 1960 to January 14th 1961. 36 animals in all were obtained. Pituitary material was stored for from 10 days to 5 weeks before being assayed.

As the two assays were performed at different times of year, there is the possibility of a difference in the sensitivity of the two groups of mice to injected pituitary tissue. The only alternative was to store the pituitaries for different periods of time. The danger of inactivation of gonadotrophins after so prolonged a period of storage had to be considered. There was also the danger of accidental damage. One entire winter collection of pituitaries intended for assay was lost because of failure of the deep freeze. (cp. Lamond, Radford and Wallace, 1959).

Virgin female albino mice, 19 to 21 days old, were allocated at random to two groups. The control group received 0.05 ml. of sterile saline daily for 3 days, the experimental group 0.05 ml. of saline pituitary extract. All injections were subcutaneous and given between 11 a.m. and 12 noon.

All mice were killed with coal gas approximately 72 hours after the first injection. The uterus, minus the fallopian tubes, was dissected out, blotted briefly on filter paper to remove fluid and was weighed on a torsion balance to 0.1 mg.

The variance of uterine weights for the mice treated with summer pituitary extract was significantly greater than that

Table XVII. Comparison of Mean Organ Weights between summer and winter animals whose pituitaries were assayed.

	Summer 1960	Winter 1960-61
Number of animals	20	36
Total weight of their pituitaries (mg.)	44.1	36.2
Average weight of their pituitaries (mg.)	2.2	1.0
Body Weight (g.)	33.55 ± 2.62	19.58 ± 0.58
Testes (mg.)	555.50 ±16.82	15.78 ± 2.24
Seminal vesicles (mg.)	189.90 ±15.36	1.19 ± 0.87
Ventral prostates (mg.)	34.13 ± 3.36	3.07 ± 0.71
Adrenal (mg.)	7.30 ± 0.85	5.96 ± 0.30
Thyroid (mg.)	2.10 ± 0.12	1.56 ± 0.10

Table XVIII. Comparison between Winter Males: mean organ weights ± Standard error

	1959-60 + 1956-57	1960-61 (Assay)	P
Body weight (g.)	19.58 ± 0.58	18.92 ± 0.62	n.s.
Testes (mg.)	15.78 ± 2.24	16.62 ± 6.43	n.s.
Seminal vesicles (mg.)	1.19 ± 0.87	0.77 ± 0.20	n.s.
Ventral prostates (mg.)	3.07 ± 0.71	5.05 ± 1.04	n.s.
Adrenals (mg.)	5.96 ± 0.30	5.30 ± 0.19	0.1 - 0.05
Thyroids (mg.)	1.56 ± 0.10	1.53 ± 0.05	n.s.

	Size of samples	
Body weight	36	25
Testes	36	25
Seminal vesicles	36	21
Ventral prostates	33	19
Adrenal	36	25
Thyroid	34	21

for control mice. Therefore, all uterine weights have been transformed into their logarithms for the analysis of differences between means (Bailey, 1959).

b. Results

The animals from which the two groups of pituitaries for assay were obtained show striking differences in their degree of sexual development (Table XVII).

The mean weights of the testes, seminal vesicles and ventral prostates of the males collected in winter as the source of pituitaries for assay, do not differ significantly from their mean weight in winter males whose pituitaries have been shown to contain vesiculated gonadotrophs. (Table XVIII).

Similarly, the mean weight of the testes of the summer males whose pituitaries were assayed does not differ significantly from the mean weight of testes in summer males shown to have well granulated gonadotrophs in their pituitaries (Table IV). The lower weight of seminal vesicles and ventral prostates in this summer, as compared with the summer of 1959 (Table IV), cannot be explained, but there is no doubt that the males whose pituitaries were used for bioassay were sexually well developed and it is reasonable to assert that they were fertile.

The mean of the logarithm of uterine weight of the mice injected with the summer pituitary extract is greater than that of their controls. The difference is statistically

Table XIX. Assay of pituitaries of summer and winter field voles

	Number of animals	Mean of Log weight of uteri of assay mice ± S.E.		P
		Pituitary extract	Saline	
Summer	5	1.2937 ± 0.0598	0.8292 ± 0.0692	< 0.001
Winter	4	0.8767 ± 0.0187	0.9504 ± 0.0203	0.05 - 0.02
P		< 0.001	0.2 - 0.1	

highly significant. (Table XIX).

The lower mean log uterine weight of mice injected with the extract of winter vole pituitaries as compared with their controls is statistically significant.

The difference in mean log uterine weight between mice injected with an extract of summer and of winter vole pituitaries is statistically highly significant.

There is no statistically significant difference between the mean log uterine weights of control mice injected with saline in July and January.

c. Discussion

From the pituitaries of summer sexually active male voles a hormone was obtained able to produce uterine enlargement in immature mice. An extract of an equal weight of pituitary tissue from winter males caused no such enlargement.

The two batches of pituitaries had been prepared for assay in an identical manner and it is most unlikely that hormone had been inactivated in the winter pituitaries and not in the summer ones.

In fact, the immature mice receiving the extract of winter vole pituitaries have a lower mean log uterine weight than their controls. The difference is statistically significant, but the number of animals involved is small and it is doubtful whether any physiological significance should at present be attached to this result. It is clearly a less striking change

than that brought about by the extract of summer pituitaries.

The possibility of a difference in sensitivity of the two groups of mice has already been mentioned. However, the mean uterine weights of the control groups in summer and in winter showed no significant difference. Laboratory mice are, moreover, maintained under fairly constant conditions throughout the year. Finally the magnitude of the difference in response suggests a real difference of physiological significance in the hormone contents of the winter and summer vole pituitary extracts.

(3) Summary of evidence pertaining to seasonal change in adenohypophyseal function.

The gonadotrophs of breeding season voles are very different in structure from those of non-breeding animals. There is also evidence of a striking difference in pituitary content of gonadotrophic hormone in the breeding and non-breeding seasons. This will be discussed further in connection with the control of breeding seasons. No striking change with season is apparent in the thyrotrophs of the vole. This might be anticipated from the lack of any marked seasonal change in the thyroid itself. The red cells are strikingly well developed in pregnant and lactating females. This suggests that they are the source of prolactin. The round yellow cells appear to be reduced in numbers in pregnant and lactating females. There is no further evidence of seasonal change in the round yellow cells and no evidence of seasonal change in the large oval cells.

D. Discussion of the internal factors controlling vole breeding seasons: the role of the adenohypophysis.

The external factors, such as light and temperature, which are involved in the control of vole breeding seasons, have already been discussed (p. 36 ff).

A striking seasonal change has been observed in the gonadotrophs of field voles. In summer breeding animals they are granulated. In winter non-breeding animals they are vesiculated.

This confirms the findings of Clarke (1957). In mature but sexually inactive males, which had received only 6 hours of light daily, complete absence of spermatogenesis was correlated with the presence in the pituitary of vesiculated PAS positive cells.

From the results of the experiments in which exogenous gonadotrophins were injected into sexually inactive voles, it has been tentatively concluded that their gonads were competent to respond. It follows that sexual inactivity in winter can be ascribed to low levels of circulating gonadotrophin. This suggests that the vesiculated gonadotrophs are not secreting gonadotrophins or are secreting them at very low levels. The results of the bioassay suggest that vesiculated gonadotrophs do not store gonadotrophin.

Vesiculated gonadotrophs occur in both regressed and inhibited males and parous and non-parous females. Thus it is the present reproductive state of an animal, which is

reflected in its pituitary cytology. Apparently no residual effect of past reproductive activity can be detected.

The males of the 1958-59 winter were well developed sexually. Their testes either contained sperm or were in advanced spermatogenesis. The pituitaries of these animals contain, in general, both granulated and vesiculated gonadotrophs and were presumably producing a certain amount of gonadotrophin, sufficient to stimulate spermatogenesis or to maintain a degree of activity in the seminiferous epithelium. In one male, however, a testis with sperm was associated with a pituitary in which apparently all the gonadotrophs were vesiculated, and therefore, presumably, neither synthesising nor secreting gonadotrophin. When an animal is hypophysectomized the involution of its reproductive organs occupies a period of days. In the rat size decrease and softening of the testis becomes evident on the 5th day after hypophysectomy (Smith, 1939). In the ferret there is little change either in testis weight or histology at 7 days. At 14 days atrophy is appreciable. In this species the experimental regression in the reproduction tract which results from hypophysectomy is about three times as rapid as the normal decline into sexual inactivity at the end of the breeding season (Hill and Parkes, 1933a). The male vole with sperm and vesiculated gonadotrophs presumably illustrates the time lag between the cessation of adenohypophyseal activity and gonadal atrophy.

The females in the winter of 1958-59 were not detectably different from the females of other winters in uterine weight, ovarian weight and ovarian histology. However, many of their pituitaries did contain a high proportion of granulated gonadotrophs, suggesting that they were not so deep in anoestrum as is usual in winter.

Considerable variation has been noted in the intensity of the PAS reaction and density of granulation in the pregnant and lactating females obtained from the field. In the number of animals available these variations could not be related to stage of pregnancy. However, their significance may lie in the changes in the pituitary content of gonadotrophin occurring in relation to pregnancy and lactation. Ladman and Runner (1953) have studied storage and release of gonadotrophins by the adenohypophysis of the mouse during gestation. They found that, while FSH production seemed to remain quite constant, LH secretion showed two periods of maximum release, one in the early and one in the late stages of pregnancy. In mid-pregnancy there was a rise in the gonadotrophic potency, indicating that the rate of hormone manufacture exceeded the rate of release at this stage.

The work of Ladman and Runner illustrates not only the type of changes in hormone secretion and hormone content which may be expected in relation to pregnancy, but also that the hormone content of the pituitary at any time will be determined

by the relationship between synthesis and secretion. This is an important consideration in interpreting the results of bioassay.

The striking seasonal change in the gonadotrophin producing cells of the vole's adenohypophysis may not be peculiar to this animal. Pomerat (1942) investigated the effects of continuous light and continuous darkness on the pituitaries and ovaries of rats. He found cells described as resembling castration cells in the adenohypophyses of rats submitted to continuous darkness. These animals had smaller ovaries than the controls. Unfortunately no photographs or drawings of the adenohypophyseal cells were published. However, the inactive vesiculated cells of the winter vole pituitary do slightly resemble vole gonadotrophs at one stage of their transformation into castration cells. Of course the gonadotrophs of a castrated animal are in a highly active state (Greep and Chester Jones, 1950), but it seems possible that the cells which Pomerat observed in rats kept in continuous darkness were of the same type as the vesiculated gonadotrophs described here for the winter vole.

Seasonal cytological changes have also been observed in the cells in the ewe's adenohypophysis which are thought to secrete gonadotrophin (Clarke, 1961). In the anoestrum gonadotrophs contained abundant granules, while in the breeding season most of the granules had disappeared. No vesiculated

gonadotrophs were seen by Clarke in the sheep.

This in itself might suggest differences between ewes and voles, both seasonally breeding animals, in the causation and depth of their anoestrum. Bioassay studies add weight to the suggestion that the annual physiological changes undergone by the pituitary of various seasonally breeding mammal species are different.

It has been seen in the present investigation that the absence of reproductive activity in winter voles is associated with low pituitary content of gonadotrophin.

On the other hand, Warwick (1946), Lamond, Radford and Wallace (1959) and Hutchinson and Robertson (1960) have found no important seasonal variation in gonadotrophic hormone content of adenohypophyseal tissue from ewes. Warwick (1946) and Lamond et al. (1959) investigated total gonadotrophin content. Hutchinson and Robertson (1960) attempted to investigate FSH and LH separately.

However, Lamond (1960) published indirect evidence suggesting a lowering of endogenous ovulation inducing hormone in anoestrus Merino ewes. He injected oestrus and anoestrus ewes with progesterone, then PMS and finally HCG. This procedure is known to cause ovulation in sheep. At low, but not at high dosages of HCG, the interval between its injection and the occurrence of ovulation was greater for anoestrus than for breeding season ewes. Lamond thought that

there might be a substance in the ewes which could act in conjunction with HCG to induce ovulation. This substance, he supposed, is present in smaller quantities in the anoestrum than in the breeding season. It could conceivably be LH. These results fit in with the view of Nalbandov (1958) that in the ewe the oestrus and anoestrus periods are characterized by different ratios of FSH to LH secretion. Fiske (1939) came to a conclusion similar in principle to Nalbandov's after studying rats in continuous light and continuous darkness. In this case the pituitaries of rats kept in the dark contained more LH than those in continuous light.

Elder and Finerty (1943) found that the pituitaries of female cotton rabbits, Sylvelagus floridanus mearnsi, maintained a relatively constant gonadotrophic potency throughout the year with a tendency to a winter increase. In the male there was, on the other hand, a high gonadotrophic potency per unit weight of pituitary tissue in the summer breeding season and low potency from October to January.

Wells (1938) found that in male ground squirrels, Citellus tridecemlineatus, gonadotrophic potency was high in males with sperm and very low, but not absent, during the long non-breeding and hibernating period.

CHAPTER IV. THE ADRENAL CORTEX

The adrenal cortex of the immature mouse contains a zone of cells next to the medulla which has been termed the X zone (Howard-Miller, 1927; Deansley, 1928). In the male this zone disappears at puberty. It is absent from the mature male mouse. In the virgin female mouse the X zone disappears only slowly, but it regresses very rapidly during the first pregnancy. In castrated male mice the X zone reappears. It involutes again if androgen is injected into male castrates (Chester Jones, 1949, 1957).

Delost and Delost (1955) report a morphologically similar zone in the adrenal of Microtus agrestis. It is absent from sexually active males, but is present in castrate males and in sexually inactive males in winter. In pregnant females, by contrast with the mouse, the zone does not regress, but is particularly well developed. The variations in the weight of the adrenal, which are found in relation to the animal's sex and its reproductive condition, can be at least partially attributed to changes in the X zone.

Clarke (1957) found in laboratory experiments that the adrenals of sexually developed male voles are lighter than those of sexually inhibited males. The latter show a region of large vacuoles next to the medulla, suggesting a degenerating X zone, while the former have no X zone.

Delost (1956b) has studied the development of the adrenal in Microtus arvalis and its variation in relation to reproductive changes. This species has an X zone which behaves similarly to that of Microtus agrestis. He has also investigated the effects of castration and hormone injection on the X zone in this species, in an attempt to determine how the X zone is controlled.

A study of field populations of the vole led D. Chitty (1952) to postulate that intraspecific strife is of great importance in the control of vole population size. As part of an investigation of the consequences of this hypothesis, Clarke (1953) studied the physiological effects of fighting on individual voles. He showed that, as a result of attacks made on strange voles by a resident pair, the adrenals of the attacked vole increase significantly in weight. It thus seemed probable that adrenal weight changes might be associated with changes in population size. H. Chitty (1961) has studied the adrenal weights of field animals collected over a six year period in Wales. No clear relationship between population size and adrenal weight could be found, although there was some evidence that the adrenals of females were heavier in an expanding population than in one which was at a peak or declining. A marked seasonal variation in adrenal weight was, however, noted. It reached a maximum in June and was lower in both spring and autumn. Immature as well as mature animals showed this fluctuation, which was, therefore, considered to be not simply a result of gonadal

activity.

In this present study the adrenals of animals obtained from the field have been weighed and examined histologically, with the object of investigating further the nature of the seasonal changes which the adrenal undergoes. It is clear that the X zone shows marked changes in relation to reproductive activity and in this connection the adrenals of castrated males have been studied. The other adrenal zones have also been examined histologically.

A. Seasonal Changes in the Adrenal

(1) Weight data

Adrenal weight, in general, increases with increasing body weight. It is, therefore, necessary in comparing the mean adrenal weights of different groups to take into account any differences in mean body weight.

a. Males

The mean weight of the adrenals of males in summer is heavier than in winter, but summer males have a much higher mean body weight.

An attempt has been made to determine the nature of the relationship between adrenal weight and body weight. The methods used have been the same as those used to investigate the relationship between thyroid and body weight. The regression of adrenal weight upon body weight has been calculated for summer and for

Table XX. Regression of adrenal weight upon body weight.

Probabilities that b differs from zero given by:

* P 0.05 - 0.01

** P 0.01 - 0.001

*** P < 0.001

	Number of animals	a	b	
Males				
Summer	37	3.0113	0.1302 ± 0.0399**	P < 0.001
Winter	65	-2.4571	0.4195 ± 0.0433***	
Females				
Winter				
Non-parous	11	2.1765	0.1815 ± 0.1144	n.s.
Parous	13	-1.4447	0.4252 ± 0.1379*	
Overall	24	-1.5258	0.4176 ± 0.0724***	

winter males. The values of a and b the constants of the equation

$$y = a + bx \qquad \text{where } y = \text{adrenal weight}, \; x = \text{body weight}$$

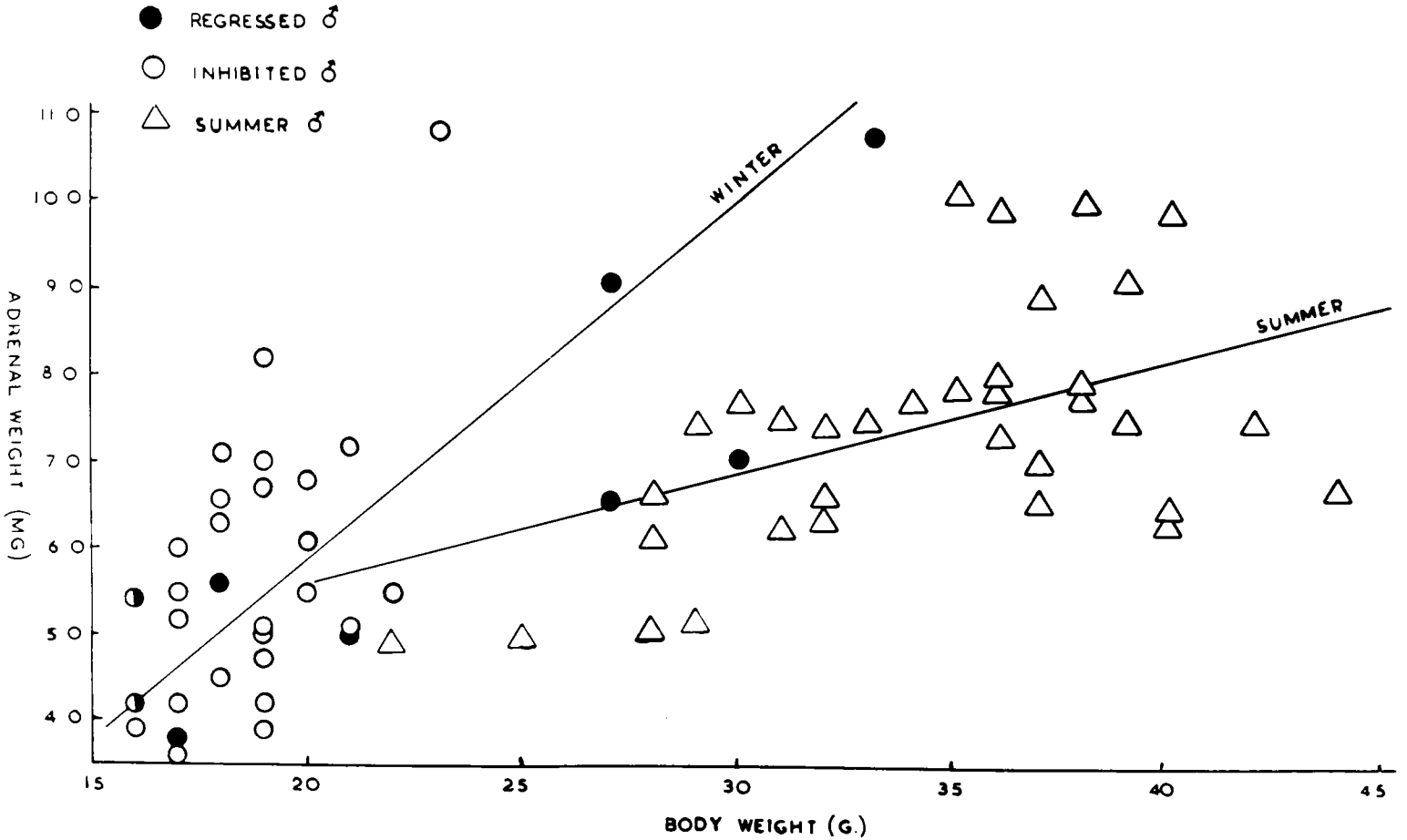
are given in Table XX.

For both summer and winter adrenals the value of b, the slope of the curve, is significantly different from zero. The difference between the summer and winter values of b is also statistically significant.

Adrenal weight and body weight do thus bear a different relationship to each other in summer and winter, suggesting a seasonal difference in adrenal physiology. For a given body weight over 20 g., adrenals are apparently heavier in winter than in summer (Text - Fig. 3).

There is no significant difference in the mean weight of adrenals of regressed and inhibited males in winter. (Table III). The difference in their body weights is on the borderline of statistical significance ($P = 0.1 - 0.05$). There is insufficient data available on regressed males for the relationship between their body weight and adrenal weight to be examined by means of a regression analysis. A plot of adrenal weight against body weight for regressed and inhibited males in winter, however, suggests that adrenal weight and body weight bear the same relationship to one another in the two groups (Text - Fig. 3).

The males in the 1958-59 winter showed considerable evidence of being sexually active. The data on their adrenal weights



Text - Figure 3. The relationship between adrenal weight and body weight in male voles obtained from the field in winter and summer: observed data and calculated regression lines.

has, therefore, been considered separately from that on males in the other winters (Table III). The males caught in the winter of 1958-59 have been separated into two groups:

1. Those with testes which weigh more than 100 mg. and contain sperm.
2. Those with testes which weigh less than 100 mg. and contain mainly spermatids.

The mean weight of adrenals of males in the first group is less than that of males in the second group, though the difference is not statistically significant.

The calculated curve (Text - Fig. 3) showing the relationship between body weight and adrenal weight in summer males, indicates that a summer male of 22g. body weight would be expected to have an adrenal weight of about 6 mg. This is in good agreement with the mean adrenal weight of those 1958-59 winter males which were sexually developed (testes > 100 mg.) and had a mean body weight of 22.6 g.

There is close correspondence in mean body weight between regressed males in winter and males with testes greater than 100 mg. in the 1958-59 winter. The mean adrenal weight is slightly heavier in the former group, but the difference is not statistically significant.

Similarly, the two groups: inhibited males in winter and males with testes less than 100 mg. in the 1958-59 winter correspond closely in mean body weight. The mean adrenal weight

of the latter group is the heavier, but the difference is not statistically significant.

b. Females

A comparison of winter and summer female adrenal weights shows that the mean adrenal weight is significantly heavier in summer (Table V). Body weight differences cannot account for this. The mean body weights of winter parous females and of summer females in their first pregnancy are about equivalent, but the adrenals of the latter are considerably heavier (Table V).

Many summer females were pregnant and their body weight includes the weight of foetuses. It was not, therefore, considered valid to treat the data on summer females by means of a regression analysis of adrenal weight on body weight.

The adrenals of primipara are lighter than those of multipara. The body weight of primipara is also less (Table V). The difference in adrenal weight is not statistically significant; that in body weight is highly significant statistically.

The numbers obtained were too few and the stage of pregnancy too imprecisely determined for an investigation of the possible influence of stage of pregnancy on adrenal weight.

The mean weight of adrenals of parous winter females is heavier than that of non-parous females in winter (Table V). The difference is statistically highly significant. Plotting adrenal weight against body weight for females in winter, however, suggests that the difference in body weight between

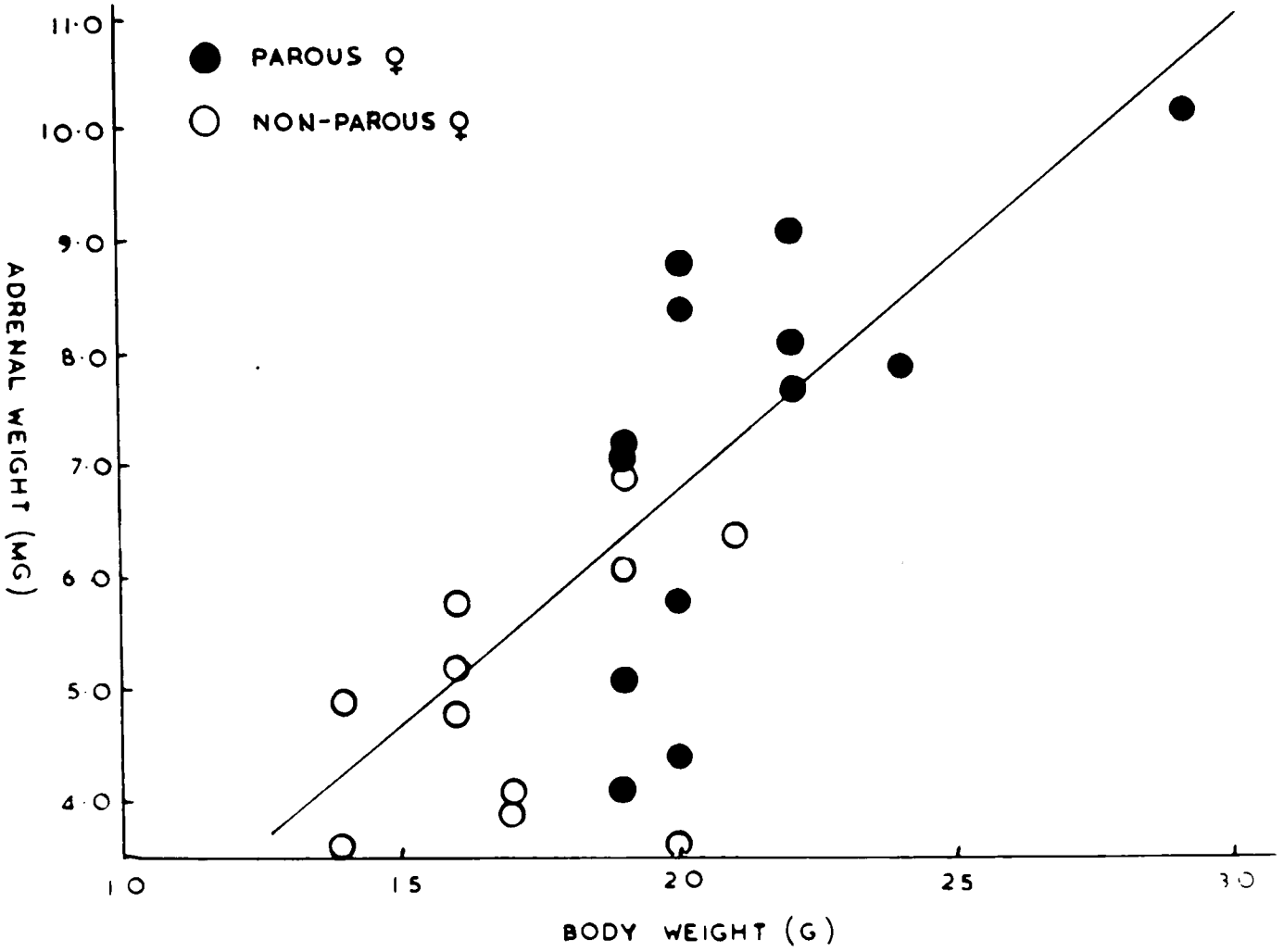
the two groups may account for their adrenal weight difference (Text - Fig. 4). This suggestion is supported by an analysis of the regression of adrenal weight on body weight for parous and non-parous females (Table XX). The slope of the regression line for non-parous females does not differ significantly from zero; that for parous females does differ significantly from zero. The difference between the slopes of the regression lines for the two groups is not statistically significant. If the data for parous and non-parous females is considered together, an overall regression line is obtained whose slope differs significantly from zero (Table XX and Text - Fig. 4).

c. Males and Females

There are sex, as well as seasonal, differences in the weights of vole adrenals.

In summer the mean weight of adrenals of females, whether primipara or multipara, is much heavier ($P < 0.001$) than that of males, even though males have a much higher mean body weight (Tables III and V).

In winter the adrenal weights of males and females are more nearly comparable. The mean weight of male adrenals is still somewhat lighter than that of females, but the difference is not statistically significant (Table V). Moreover, there is no statistically significant difference in the slopes of the regression lines relating adrenal weight to body weight of males and females in winter (Table XX).



Text - Figure 4. The relationship between adrenal weight and body weight in female voles obtained from the field in winter: observed data and calculated overall regression line.

(2) Histology of the adrenal cortex

The terminology of Nicander (1952) has been adopted.

The adrenal cortex of the vole shows in transverse section:

1. A zona glomerulosa, with cells arranged in groups, often in a loop formation. Each group is outlined in connective tissue, but connective tissue fibres are not evident between the cells of a group. The cells have a tendency to be flattened tangentially. The size and depth of staining of the nuclei is variable. Differences of this kind must, however, be interpreted with caution, as handling of unfixed material appears to result in a superficial zone of pycnotic nuclei. The zona glomerulosa usually contains few lipid droplets.

2. An intermediary zone, which is very variable in its degree of development, but can usually be distinguished as a band of small square or very thin cells, with small dark nuclei, separating the zona glomerulosa from the zona fasciculata.

3. A zona fasciculata: its cells are arranged in radially disposed cords. They are the largest cells in the adrenal cortex, with large, pale staining, vesicular nuclei. The cells contain a variable number of fine lipid droplets, whose removal from paraffin sections gives the cytoplasm a foamy appearance. Sometimes it is possible to distinguish an outer and an inner zone in the fasciculata. When such a distinction can be drawn, it is the outer fasciculata which has the larger cells, with larger and more palely staining nuclei and more lipid droplets. There is usually a gradation, rather than a definite boundary,

between the two zones.

4, A distinct juxtamedullary zone may be present. Its appearance is very variable. The term "X zone" has been applied by Delost and Delost (1955) to what is here called the juxtamedullary zone. It has seemed preferable to employ the non-committal term, juxtamedullary zone. Later (p.134 ff) the possible relationship between this zone and the X zone of the mouse is discussed.

There does not seem to be a zona reticularis in the adrenal cortex of the vole.

There are seasonal changes in all zones of the adrenal cortex, but the most striking changes take place in the juxtamedullary zone. These changes will be described first.

a. The Juxtamedullary Zone

(i) Summer males

The zone is present in 11 of 14 summer males examined as a few rows of cells with scanty, pale staining cytoplasm, devoid of lipid droplets, and with pycnotic flattened nuclei (Plate X, Fig. 1). In this area application of the PAS method reveals abundant connective tissue. In 3 males there is no juxtamedullary zone (Plate X , Fig. 2). The columns of cells of the zona fasciculata appear in sections stained with Ehrlich's haematoxylin and eosin or coloured with Sudan Black to abut directly onto the medulla. However, PAS staining shows that connective tissue forms a rather incomplete sheath around the

medulla, separating it from the cells of the zona fasciculata.

(ii) Winter males (excluding males of 1958-59 winter)

The juxtamedullary zone is present in all winter males. It consists of small cells with pycnotic, irregularly shaped nuclei. Blood lacunae are conspicuous between the cells, which often become increasingly flattened near the medulla. All the gelatine embedded winter male adrenals coloured with Sudan Black show the presence of lipid droplets in the juxtamedullary zone. Their concentration here is as high or higher than in the other three adrenal cortical zones (Plate X , Fig. 4). In about half the inhibited males examined, the juxtamedullary zone contains large vacuoles, showing as circular spaces in paraffin sections (Plate X , Fig. 3). The contents of these vacuoles may colour with Sudan Black, but does not invariably do so. In sections of the adrenals of both inhibited and regressed males stained with PAS, connective tissue is seen to be particularly striking in the juxtamedullary region. It may tend to form a definite sheath around the medulla in both inhibited and regressed males (Plate XI , Figs. 1, 2).

(iii) Males of 1958-59 winter

As previously described (p. 33) the males in this winter can be divided in two groups: those with a combined testis weight of over 100 mg., which had sperm in their testes; those with a combined testis weight less than 100 mg., whose testes contained mainly spermatids. It is considered that the latter

group was in process of sexual development, rather than in process of regression.

Males with testes weighing more than 100 mg.

The juxtamedullary zone is slight or absent, as in a summer male. In only one animal did the zone form a significant part of the cortex, occupying about a third to a half of it. This particular animal had sperm in only a few tubules and very small seminal vesicles.

The one male in the 1960-61 winter with sperm in its testes had no juxtamedullary zone in the adrenal.

Males with testes weighing less than 100 mg.

The juxtamedullary zone occupies a quarter to a half of the cortex. It is similar in structure to the juxtamedullary zone of males in other winters. Large vacuoles are present, showing as circular spaces in paraffin sections, in all animals.

(iv) Summer females

The juxtamedullary zone differs in the details of its structure in primipara and in multipara.

Primipara

Careful examination may be necessary to distinguish the juxtamedullary zone from the zona fasciculata. It occupies about half the cortex. Its cells stain more intensely with eosin than do the cells of the zona fasciculata. The cell nuclei are large, but slightly irregular in outline, rather than

vesicular. The arrangement of cells is not so regular as in the zona fasciculata. The juxtamedullary zone extends to the medulla and PAS staining shows that no connective tissue sheath separates it from the medulla (Plate XII, Fig. 1, 3).

Multipara

As in primipara the juxtamedullary zone occupies about half the cortex, but it is more readily distinguishable from the zona fasciculata, having, in multipara, cells with distinctly irregular nuclei. Also in contrast with primipara, it does not extend unchanged to the medulla. Instead, its inner part consists of smaller cells, with darkly staining irregular nuclei, and separated by conspicuous blood lacunae. Connective tissue is conspicuous in this region (Plate XII, Fig. 2, 4).

In both primipara and multipara the content of lipid droplets which colour with Sudan Black is less in the juxtamedullary zone than in the zona fasciculata. Lipid droplets may, in fact, be almost absent from the juxtamedullary zone. Their numbers are not in any very obvious was related to state or stage of pregnancy. Of two females, both apparently in their first pregnancy and both at mid-term, one had a juxtamedullary zone with many lipid droplets, while in the other there were no lipid droplets.

(v) Winter females (excluding females of 1958-59 winter)

The juxtamedullary zone is present in all winter females, but it is different in appearance from the juxtamedullary zone of summer females. In 4 females, 1 non-parous and 3 parous,

it is a narrow zone of pale, flat cells with pycnotic flattened nuclei, very similar in appearance to the juxtamedullary zone of summer males (Plate X , Fig. 1). In the remaining animals it occupies a quarter to a third of the cortex and is like the juxtamedullary zone of winter males. In 3 females, 2 parous and 1 non-parous, there are blood lacunae between cells with pycnotic, oddly shaped nuclei and little cytoplasm. In 3 non-parous females the structure is very similar, but there are, in addition, large round vacuoles in paraffin sections. Connective tissue is abundant in this zone.

(vi) Females of 1958-59 winter

The juxtamedullary zone was absent from 3 non-parous females in this winter. When present it showed a similar range of structure to that seen in other winters.

b. The zona glomerulosa

(i) Summer males

The zona glomerulosa is particularly well developed. In 12 of 14 summer males examined, it is 5 to 6 cells deep. The cells are large with large, rather pale staining nuclei. In the 2 remaining animals it is about 3 to 4 cells deep with more darkly staining and smaller nuclei. The zona glomerulosa of summer males is unusual in containing large numbers of lipid droplets. All sections of summer male adrenals which were embedded in gelatine and coloured with Sudan Black show that the zona glomerulosa contains lipid droplets in the same considerable

concentrations which are present in the cells of the zona fasciculata (Plate X , Figs. 1, 2).

(ii) Winter males (excluding males of 1958-59 winter)

The development of the zona glomerulosa is rather variable. It is usually about 3 to 4 cells deep, but the cells may be large, with large, pale staining nuclei or they may be smaller, with smaller, more darkly staining nuclei. Lipid droplets are few or absent.

(iii) Males of 1958-59 winter

With testes weighing more than 100 mg.

The zona glomerulosa is about 4 cells deep. The cells may be large with large pale staining nuclei and evidence of lipid droplets in the cytoplasm or they may be smaller and have more darkly staining nuclei.

With testes weighing less than 100 mg.

The zona glomerulosa is 3 to 4 cells deep. The cells tend to be rather small with moderately darkly staining nuclei.

(iv) Summer females

Primipara

The zona glomerulosa is a zone of small cells and is about 4 cells deep.

Multipara

The zona glomerulosa tends to be a wider zone in multipara than in primipara. It is still about 4 cells deep, but the cells are larger.

(v) Winter females

It is similar in structure to the same zone in winter males.

c. The intermediary zone

(i) Summer males

The zone is present, but not strikingly well defined. It contains lipid droplets.

(ii) Winter males (excluding males of 1958-59)

In all the regressed males and in about 50% of the inhibited males examined, the intermediary zone forms a distinct and easily recognized layer of flattened cells. In the remaining inhibited males it is present, but not strikingly well developed. In all animals examined lipid droplets are few or absent.

(iii) Males of 1958-59 winter

Males with testes weighing more than 100 mg.

The intermediary zone is present in all, striking in two animals.

Males with testes weighing less than 100 mg.

The intermediary zone is very variable in its development: not obvious in 2 animals, present in 2 and distinct in 2.

(iv) Summer females

The intermediary zone is not conspicuous in either primipara or multipara.

(v) Winter females

The intermediary zone is recognizable in all winter females

and is striking in more than half of those examined.

d. The zona fasciculata

(i) Summer males

This zone is well developed. A difference between the inner and outer zona fasciculata is distinguishable in 11 of 14 summer males.

(ii) Winter males (excluding males of 1958-59 winter)

The zona fasciculata shows the usual large cells with vesicular nuclei and variable numbers of lipid droplets. Inner and outer zones could not be distinguished, However, particularly in the winter of 1960-61, there was a tendency for a patchy appearance in the zona fasciculata. Groups or columns of cells had more darkly staining nuclei and fewer lipid droplets than cells in the remainder of the zone.

(iii) Summer females

The zona fasciculata consists of large cells, with large, vesicular nuclei and abundant lipid droplets. No subdivision into inner and outer zones can be detected.

(iv) Winter females

The zona fasciculata consists of cells with pale staining, vesicular nuclei and with variable numbers of lipid droplets. The tendency to a patchy appearance, noted for the males of the 1960-61 winter, was not seen in any winter female.

B. The Effects of Castration on the Adrenals

(1) Methods

The technique of castration has been described (p. 42).

Immediately after death the paired adrenals of control and experimental animals were removed and weighed fresh on a torsion balance to 0.1 mg. They were fixed in either formal sublimate or Bouin's fixative (Appendix I).

They were prepared for histological examination as previously described (p. 14).

(2) Results

a. Weight data

Castration results in a statistically significant increase in the weight of vole adrenals (Table VII).

b. Histology

The juxtamedullary zone

It is in this zone that the changes associated with castration take place.

In eight control animals, as in a sexually developed male in the field, there is little or no juxtamedullary zone.

In those of their litter mates which had been castrated for 21 days or more, a juxtamedullary zone can be recognized. It occupies a quarter to a half of the cortex. Its cells are smaller than those of the fasciculata. They have nuclei which stain rather more darkly than the nuclei of the zona fasciculata

cells and which are also somewhat irregular in outline. The regular cell arrangement seen in the zona fasciculata is interrupted and replaced by a tendency to anastomoses between cell cords. There are blood lacunae between the cell cords (Plate XI, Fig. 3). Lipid droplets are few or absent.

Two animals which had been castrated for less than 21 days had no juxtamedullary zone.

In no experimental animal was the juxtamedullary zone as well defined as in a male or female captured in winter in the field.

In the three remaining control animals a juxtamedullary zone is probably present. The nuclei in the inner third of the cortex are very slightly irregular in outline. The cytoplasm appears to be more intensely stained, but the regular cell arrangement of the zona fasciculata persists.

Of the experimental animals which correspond with these controls, one, which had been castrated for 56 days, has a juxtamedullary zone occupying about half the cortex. The cell nuclei of this zone are only slightly irregular in outline. In another, castrated for 112 days, there are a few large vacuoles in a juxtamedullary zone occupying about a quarter of the cortex. In a third, castrated for 84 days, the juxtamedullary zone occupies a third to a half of the cortex and is packed with large vacuoles (Plate XI, Fig. 4).

Zona glomerulosa

Castration produces no obvious change in this zone. It is rather variable in appearance in both control and experimental animals. It is about 3 to 5 cells deep, the cells being in the main rather small. Their nuclei are more or less darkly staining. In none of these laboratory animals is the zona glomerulosa as well developed as in males obtained from the field in summer. This agrees with the observation of Delost and Delost (1955).

Intermediary zone

It can be distinguished in all control and experimental animals and is striking in about 50% of animals from both control and experimental groups.

Zona fasciculata

This is generally well developed.

C. Discussion

(1) Seasonal Changes in the Adrenal

The vole adrenal shows weight changes with season. In the summer there are also sex differences in adrenal weight. It will be seen that reproductive activity is an important factor in producing these weight differences.

There is close agreement between the weight data reported here and that given for Microtus agrestis by Delost and Delost

(1955) and for Microtus arvalis by Delost (1956b). It has been confirmed that pregnant females have heavier adrenals than sexually active males and that castration results in an increase in the weight of male adrenals. In the winter, male and female adrenals are more nearly equal in weight. The mean adrenal weight of male Microtus is greater in summer than in winter. The adrenals of pregnant females are much heavier than those of winter females.

In general, organ weight increases with increasing body weight. It is, therefore, important to consider any differences in body weight in assessing the possible significance of differences in mean adrenal weight between groups. In the two papers by Delost on Microtus spp. the mean adrenal weight for each individual has been calculated and it is the mean of these mean adrenal weights which is presented. No standard error of the final mean is given and no indication of the mean body weight of each category considered.

The relationship between adrenal weight and body weight in summer and winter males has been investigated by means of a regression analysis. The slopes of the two regression lines differ significantly. Although it must be admitted that there is little overlap in body weight between the two groups, there is a suggestion that the relationship between the adrenal weight and the body weight of males is different in summer and in winter, indicating a seasonal difference in adrenal physiology.

Moreover, although the mean adrenal weight of males is lower in winter than in summer, when body weight differences are taken into consideration by means of a regression analysis, the indication is that, for males of a given body weight over 20 g., the adrenal is heavier in winter than in summer (p.114-5).

Reproductive sub-groups have been recognized within seasons and adrenal weight differences between the sub-groups have been investigated. In fact, it seems that adrenal weight and body weight bear the same relationship to one another in regressed and inhibited males and in parous and non-parous females. There is a difference in adrenal weight between primipara and multipara which may or may not be related to their difference in body weight. If there is a real adrenal weight difference between the two groups, it might be explicable in terms of the probable age difference between animals in the two groups (p. 35) or to the effect of previous pregnancies in multipara, and the absence of such an effect in primipara. The males of the 1958-59 winter show evidence of sexual development and differ in adrenal weight from males of other winters, though the differences are not statistically significant. The adrenal weight of these various categories will be discussed further in relation to their adrenal histology.

All zones of the adrenal cortex show seasonal variation in their cytology and histology. The most striking changes occur in the juxtamedullary zone. It is evident that weight differences can be at least partially explained on the basis of changes in this zone.

The lower relative weight of summer, as compared with winter, male adrenals is associated with a virtual absence of any juxtamedullary zone in the adrenals of summer males. Winter males in a non-breeding condition have a distinct juxtamedullary zone, occupying about a quarter to a third of the cortex.

The absence of such a zone is clearly correlated with the completion of sexual development, since the winter males whose testes contain sperm have little or no juxtamedullary zone. On the other hand, a juxtamedullary zone is present in the adrenal cortex of those males, caught in the winter of 1958-59, whose testes contain mainly spermatids. These males were probably developing sexually, rather than regressing (see p. 38). The juxtamedullary zone in these animals contains a variable number of large vacuoles, showing as circular spaces in paraffin sections.

Two sets of circumstances have been recognized in which the juxtamedullary zone may appear in an adrenal from which it was previously absent. A juxtamedullary zone is present in both regressed and inhibited males in winter. In a male which has been fully developed sexually it must, therefore, be reformed when regression of the reproductive system occurs at the end of the breeding season.

A juxtamedullary zone also appears in the adrenals of males which have been castrated. In the experiments reported here the testes have been removed from eleven sexually mature,

laboratory bred males. There is little or no juxtamedullary zone in the adrenal of eight control animals. In their litter mates, which had been castrated for 21 days or more, a juxtamedullary zone occupies a quarter to a half of the cortex. This juxtamedullary zone is not, however, so readily distinguishable from the zona fasciculata as in a winter male.

Delost and Delost (1955) obtained similar results in Microtus agrestis. The adrenals of sexually developed control males contained no "X zone". In males which had been castrated for 21 days or more an "X zone" was present.and occupied a quarter to a third of the cortex. Numbers of vacuoles were a feature of this zone.

The juxtamedullary zone resembles the X zone of the male mouse in being generally absent from mature, sexually developed males and in reappearing in such males after castration. It seems reasonable to assume an homology.

The recognition of the juxtamedullary zone of male voles as a discrete zone of the adrenal cortex depends on a number of features. The nuclei of the cells of the juxtamedullary zone are more or less irregular in outline. This irregularity may be slight or the nuclei may be very mis-shapen and pycnotic. The cytoplasm of juxtamedullary zone cells appears to stain more intensely than does the cytoplasm of zona fasciculata cells. The regular arrangement of cells seen in the zona fasciculata is to a greater or lesser extent interrupted in the juxtamedullary

zone. It tends to be replaced by a more reticular arrangement. There may be wide blood lacunae between the cell cords of the juxtamedullary zone.

In a winter female a juxtamedullary zone with these features can be recognized.

In the summer females, which were all either pregnant or lactating, approximately half the cortex is occupied by a juxtamedullary zone. This is no doubt the source of the high mean weight of summer female adrenals.

Careful examination shows the juxtamedullary zone to differ from the zona fasciculata in having cells with slightly irregular nuclei, more intensely stained cytoplasm and less regular arrangement. It has these features in common with the other juxtamedullary zones described and is, therefore, presumably also an X zone.

In a female mouse which has been pregnant, no X zone is present. The X zone disappears, not at puberty, as in the male mouse, but rapidly during the first pregnancy. It also disappears from virgin females, but more slowly, often by fatty degeneration (Howard-Miller, 1927; Deansley, 1928).

Delost (1956b) has examined females of Microtus arvalis from birth to 65 days of age and found no signs of degeneration of the X zone. He also examined pregnant females and found in them a large juxtamedullary zone or X zone, which he assumed to have arisen by further development of the zone seen in unmated females.

However, the examination of field specimens of Microtus agrestis by Delost and Delost (1955) showed that the X zone can disappear completely from females. Two females caught in the winter of 1958-59 confirm this. Neither shows an X zone.

More extensive data are necessary to establish whether the X zone of the immature female vole gives rise directly to the zone seen in the pregnant female and how the transformation takes place. There is one fact which suggests that the transformation may be direct. In the mouse, involution of the X zone gives rise to a connective tissue capsule around the medulla (Howard-Miller, 1927; Deansley, 1928). In primiparous females, obtained from the field in summer, there was no evidence of connective tissue separating the X zone from the medulla.

Further investigation is also necessary to establish whether size of litter or stage of pregnancy affects the X zone. It is not known whether, or to what extent, the X zone is modified between pregnancies. It has been noted that the structure of the X zone is different in primipara and in multipara, showing that some change must take place at least at the end of, though not apparently during, the first pregnancy. An X zone was present and well developed in females which were lactating and had no evident foetuses in the uterus. It has, however, been pointed out that these females may have been in the first week of pregnancy.

(2) Control of the X zone

Chester Jones (1957, for discussion and references) suggests that the X zone of mice is under the dual control of LH and androgen, LH stimulating it and androgen inhibiting, either the production of LH or the X zone directly. In the female the ovary is suggested as responsible for androgen production, in small amounts in the unmated female and in greater quantities, stimulated by placental gonadotrophins, during pregnancy.

Deansley (1958) found that the X zone of unmated ovariectomized female mice and of castrate males atrophied after injections of andrenocorticotrophic hormone (ACTH). She suggests that in this case the adrenal itself is responsible for the production of the androgen which causes atrophy of the X zone. There was evidence of stimulation of the seminal vesicles and ventral prostates, further supporting this view.

Delost and Chirvan-Nia (1960) have obtained similar results, but found the type of degeneration which occurred after ACTH administration to be histologically different from that occurring normally in that no lipid vacuolization was seen.

Delost (1956b) had obtained involution of the X zone of castrate Microtus arvalis with cortisone injections, as well as with injections of testosterone.

The absence of an X zone from the adrenal cortex of sexually developed male Microtus is, clearly, associated with a high level of circulating androgen. In winter males, castrated males and

also in winter females, all of which have an X zone, it is reasonable to assume that there is little or no circulating androgen.

Castrated animals have an increased output of gonadotrophins (Greep and Chester Jones, 1950). The development in their adrenals of an X zone might well be under the influence of LH. However, the results of injection and bioassay experiments (pp. 51 and 98) have indicated that in voles in winter the amount of pituitary gonadotrophin in the circulation is minimal, so that it is difficult to imagine that the X zone has been stimulated, or is being maintained, by LH in these animals. The different structure of the X zone in castrate and in winter, sexually inactive animals, whether male or female, further suggests that the endocrine situation maintaining it is different in the two groups.

There is no information about the function of, or the factors controlling, the especially well developed X zone of pregnant females.

A vacuolar appearance has been described in female mice, associated with fatty degeneration of the X zone (Deansley, 1928). A similar appearance has been detected in the X zone of four different groups of voles:

1. In the voles, caught in the winter of 1958-59, and with testes weighing less than 100 mg. These voles were probably beginning their sexual development, rather than regressing. Since the X

zone is absent from male Microtus which show full sexual development, it would be anticipated that involution of the X zone would be occurring in these developing animals and the presence of large vacuoles can be interpreted as an aspect of involution. Their testes contain spermatocytes and spermatids. The interstitial cells are well developed, so that, although the seminal vesicles were small, androgen was presumably being produced. The higher mean weight of the adrenals of these males as compared with the males with sperm in their testes, caught in the same winter, is presumably due to the possession by the former of a degenerating X zone, absent from the males which are more fully sexually developed. In fact the mean adrenal weight of this group of developing males is quite high. It is not significantly different from the mean adrenal weight of summer males, which have a much higher mean body weight. Delost (1956b) found that adult adrenal weight was reached very early, by 15 days, in Microtus arvalis. This early attainment of adult adrenal weight, however, hides important differences between the two groups. The adrenals of young, developing males contain an X zone, which is absent from older and sexually mature males. The mean adrenal weight of the latter group is high because they are older and heavier animals, not because their adrenals contain an X zone.

2. In some inhibited males. This suggests that either involution of the X zone had begun in these animals in the autumn or

that it may proceed throughout the winter in response to very low levels of androgen production. The X zone of both regressed and inhibited males in winter colours strongly with Sudan Black. In the mouse the cells of the X zone do not show sudanophilia when undegenerate (Chester Jones, 1957). In the mouse, involution of the X zone gives rise to a connective tissue capsule around the medulla (Howard-Miller, 1927; Deansley, 1928). Holmes (1955) suggests, therefore, that a primary X zone and a medullary connective tissue capsule cannot coexist. An X zone which has arisen secondarily, for example in a castrate male, may occur with a medullary connective tissue capsule, if this has not been destroyed by phagocytosis. It might be supposed that the adrenals of regressed and inhibited males in winter could be distinguished on the basis of the presence or absence of a medullary connective tissue capsule. In fact it seems that connective tissue is abundant in the region around the medulla in both groups of males. Some involution of the X zone may have taken place in inhibited males, but theirs cannot be a true secondary X zone. If there had been, at any stage, complete involution of the primary X zone, there would also presumably have been considerable testicular development and the animals would be classified as regressed, not as inhibited.

3. In some non-parous females. It is not known under what influence regressive changes occur in the X zone of unmated female Microtus. Perhaps the hormone responsible emanates from

the ovaries (Chester Jones, 1957) or from the adrenals themselves (Deansley, 1958).

4. In two castrate males. Three of the control males used in the castration experiments, although they have heavy testes, containing sperm, and well developed accessory organs, also have an X zone in the adrenal. It occupies the inner third of the cortex. In their castrated litter mates, the zone has apparently undergone modification. The zone is similar in structure in one, but occupies about half the cortex, has a few large vacuoles in a second and is packed with large vacuoles in a third. All these animals had been castrated for 2 months or more. These facts cannot be satisfactorily explained. It is not known why an X zone should be present in the three controls in which there is every evidence of a high level of androgen secretion. It is not known under what influences the apparent regressive changes took place in the adrenals of their castrate litter mates. Perhaps the stimulus might come from the adrenal itself (Deansley, 1958). The stimulation of the ventral prostate suggests that an androgen is circulating in castrated males, but most of them appear to show no regressive changes in the X zone. It may, however, be noted that Delost and Delost (1955) described vacuoles as a constant feature of the X zone of castrate Microtus agrestis. Winter males also have a stimulated ventral prostate. Possible evidence of X zone degeneration in winter males (the presence of vacuoles and sudanophilia) has already been noted.

Not only the X zone shows seasonal changes. The zona glomerulosa is particularly well developed in summer and particularly in males, agreeing with the observation of Delost and Delost (1955). In the winter males which were sexually developed, there was no marked hypertrophy of the zona glomerulosa. Its seasonal change is, therefore, not primarily related to reproduction, but to some seasonal change in the external environment. The zona glomerulosa is not so well developed in sexually developed laboratory males as it is in summer field males.

The intermediary zone is especially prominent in winter. The fasciculata appears more active in summer. The significance of these differences is not known.

H. Chitty (1961) has found striking seasonal variation in the adrenal weight of Microtus agrestis. In mature and immature animals of both sexes there is a pattern of regular increase in adrenal weight from March to June, then a steady decline to the lowest levels in the winter. Since, in the present study, catches were made only in mid-summer and mid-winter, there are no opportunities for comparisons of the kind made by Chitty.

CONCLUSION

Seasonal changes have been detected in all those endocrine organs of the vole which have been studied. The most striking changes which occur, namely those in pituitary gonadotrophs, in one type of pituitary acidophil (the red cell), in the juxtamedullary or X zone of the adrenal cortex and in the gonads themselves, are clearly related to some aspect of the seasonal change from a breeding to a non-breeding condition. The zona glomerulosa, the intermediary zone and the zona fasciculata of the adrenal cortex also appear to undergo a seasonal change in histology, and presumably, therefore, in activity. However, these alterations do not seem to be related to reproductive activity. There is histological evidence of a tendency to greater thyroid activity in the summer, which may or may not be related to reproductive activity.

Other investigators have shown that light, and perhaps also temperature, are important proximate factors in the control of vole breeding seasons (Baker and Ranson, 1932a, 1932b, 1933; Kennedy, 1962). From the observations made on field animals, it is possible to state that these are not the sole proximate factors. There are evidently circumstances in which, despite low temperatures and short days, sexual development will occur. In one winter, voles were obtained in which there was a

considerable degree of sexual development. Examination of meteorological records showed that the months preceding the capture of these animals were neither unusually warm, nor unusually sunny. Nutrition cannot be excluded as a factor which may enable winter sexual development to occur. The state and size of the population are other variables which may be important (Chitty, 1952; Clarke, 1955).

This information about proximate factors in the control of vole breeding seasons is based on chance observations. The main part of the study was directed towards the investigation of internal changes and some idea has been gained of the alterations in endocrine function which are responsible for, or which accompany, seasonal gonadal activity.

There is suggestive evidence, from experiments in which exogenous gonadotrophins were injected, that the gonads of non-breeding voles can respond to gonadotrophins. This, combined with the results of an experiment using a bioassay procedure to measure the gonadotrophin content of pituitaries from summer and winter males, suggests that, in a winter non-breeding animal, gonadotrophin is being neither synthesised nor secreted. This cessation of synthesis and secretion is responsible, then, for the absence of sexual development in field voles in winter.

In birds there is considerable evidence that the cessation of breeding is brought about by the development of refractoriness in some part of the gonad-pituitary mechanism (Lofts and

Marshall, 1958; Wolfson, 1959). This does not seem to be the case in voles, which, if given 16 hours of light per day, will continue to breed indefinitely in captivity.

The pituitaries of breeding voles contain well granulated periodic acid-Schiff positive cells, which castration experiments have indicated as the source of gonadotrophin. In non-breeding animals the well granulated cells are replaced by vesiculated and presumably inactive gonadotrophs. Chemical thyroidectomy and the study of lactating and non-lactating female voles have provided further evidence of functional differentiation of adenohypophyseal cells.

The link between the pituitary and the gonads has been firmly established for a number of mammalian species by classical endocrinological studies. This investigation further emphasises the importance of the pituitary in mediating the changes which the reproductive organs undergo and which are apparently ultimately dependent upon changes in the external environment. The way in which the pituitary is itself influenced remains largely obscure. The hypothalamus is clearly implicated, but the precise nervous connections between receptor organs and the hypothalamus are not known. Similarly, the identity of the postulated transmitter substance, produced by the hypothalamus and carried to the pituitary via the hypophyseal portal system, remains to be discovered (see Harris, 1955). There is some evidence that neurohypophyseal hormones,

or substances resembling them, can induce secretion of adenohypophyseal hormones (e.g. adrenocorticotrophic hormone, Martini, Pecile and Giuliani, 1960).[1]

It has been noted that there occur striking seasonal changes in the juxtamedullary or X zone of the adrenal cortex and that these changes are associated with sexual activity. Further investigation will be necessary to establish the function of the X zone and how it is controlled. This zone is particularly well developed in pregnant and lactating females. This enquiry has also emphasised the possibility that the reproductive tract of the male in winter may be influenced by the adrenal cortex, though the nature and the significance of this influence remain to be discovered.

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APPENDIX I

FIXATIVE FORMULAE

Formal sublimate

40% formalin	10 ml.
saturated aqueous mercuric chloride	90 ml.

Bouin's fixative

Saturated aqueous picric acid	75 ml.
40% formalin	25 ml.
glacial acetic acid	5 ml.

Formal calcium

40% formalin	10 ml.
10% aqueous anhydrous calcium chloride	10 ml.
distilled water	80 ml.

Orth's fixative

potassium dichromate	2.4 g.
sodium sulphate	0.94 g.
40% formalin	9.5 ml.
distilled water	90.5 ml.

APPENDIX II

METHODS USED IN STAINING THE PITUITARY

A. Periodic Acid - Schiff technique.

see Purves and Griesbach 1951b.

5 μ sections stained.

Solutions:

Periodic Acid 0.5% aqueous

Schiff's solution prepared as Glick 1949

Harris's haematoxylin x 5 diluted

2% orange G in 5% phosphotungstic acid

The mercury precipitate was removed from material fixed in formal sublimate with ½% iodine in 70% alcohol. 5% thiosulphate was used to remove the iodine. After a wash in running tap water the slides were transferred to distilled water and from this to:

1. Periodic acid solution, on slide, 5 mins.
2. Short rinse in distilled water.
3. Schiff's solution, on slide, 15 mins.
4. Wash in running tap water, 20 mins.
5. Differentiate in 70% alcohol 30 mins.
6. Rinse with distilled water.
7. Nuclear stain may be added at this stage: x 5 diluted Harris's haematoxylin, 30 secs.

8. Wash in running tap water to blue.
9. Counterstain if used: phosphotungstic acid - orange G, 10 secs.
10. Differentiation in tap water, observing the process of differentiation under the microscope.

Alcohols, xylene, balsam.

B. Gomori's aldehyde fuchsin

5 μ sections stained

Solutions:

Aldehyde fuchsin prepared as Gomori, 1950.

Slides from distilled water into:

1. Lugol's solution, 1 hour.

This preliminary mild oxidation with Lugol's solution is essential if reliable staining of thyrotrophs is to be obtained.

2. Rinse with distilled water.
3. 5% thiosulphate, 2 mins.
4. Wash in running tap water, 5 mins.
5. Rinse with distilled water.
6. Aldehyde fuchsin, 1 hour.
7. Rinse with distilled water.
8. Nuclear stain and/or phosphotungstic acid - orange G as counterstain may be added, see section A.

Alcohols, xylene, balsam.

C. Mallory's trichrome stain

2 and 3 μ sections stained.

Solutions:

Harris's haematoxylin x 5 diluted

Acid fuchsin - Orange G prepared as Crossmon, 1937

Aniline blue prepared as Crossmon, 1937.

Mercury precipitate removed from formal sublimate fixed material with ½% iodine in 70% alcohol. 5% thiosulphate to remove iodine. Wash in running tap water. Slides from distilled water into:

1. x 5 diluted Harris's haematoxylin, 30 secs.
2. Blue in tap water
3. Rinse in distilled water.
4. Acid fuchsin - orange G, dip in and out, 1-5 secs.
5. Rinse in distilled water.
6. 1% phosphotungstic acid, 4 hours.
7. Aniline blue, 10 mins.
8. Rinse in distilled water.
9. 90% alcohol to differentiate.

Alcohols, xylene, balsam.

APPENDIX III. Details of Tests of Statistical Significance

Males: P values for specified comparisons

	S ♂ v. W ♂	W ♂ v. 2-3 month old laboratory stock	W regressed ♂ v. W. inhibited ♂	1958-59 W ♂ v. all other W ♂	1958-59 W ♂, Testes > 100 mg. v. 1958-59 W ♂, Testes < 100 mg.
Body weight	< 0.001	0.01-0.001	0.1-0.05	n.s.	n.s.
Testes	< 0.001	< 0.001	n.s.	0.05-0.02	0.05-0.02
Seminal vesicles	< 0.001	< 0.001	0.05-0.02	0.05-0.02	0.01-0.001
Ventral prostates	< 0.001	0.01-0.001	n.s.	-	-
Adrenal	< 0.001	n.s.	n.s.	0.1 - 0.05	n.s.
Thyroid	< 0.001	0.1 -0.05	0.1-0.05	0.1 - 0.05	-

S = summer

W = winter

APPENDIX III. continued

Females: P values for specified comparisons

	S ♀ v. W ♀	Primipara v. Multipara	Parous v. Non-parous	Non-parous v. Primipara	Parous v. Multipara
Body weight	< 0.001	<0.001	n.s.	n.s.	0.01-0.001
Ovary	< 0.001	n.s.	<0.001	< 0.001	< 0.001
Adrenal	<0.001	n.s.	<0.001	<0.001	0.01-0.001
Uterus			<0.001		
Thyroid	n.s.	n.s.	<0.001	n.s.	n.s.

Males and Females: P values for specified comparisons

	W ♂ v. W ♀	S ♂ v. S ♀
Body weight	n.s.	
Adrenal	n.s.	< 0.001
Thyroid	n.s.	0.01-0.001

PLATES

Plate I

Figure 1. Testis of a male vole, obtained from the field in summer, showing large seminiferous tubules, all stages of spermatogenesis and numerous large interstitial cells. (Formal sublimate and Ehrlich's haematoxylin and eosin, 5 μ; x 95)

Figure 2. Testis of a male vole, obtained from the field in summer, showing heavily lipoidal interstitial cells. (Formal calcium and Sudan Black, 10 μ; x 95)

Figure 3. Seminal vesicle of a male vole, obtained from the field in summer, showing high columnar epithelium. (Formal sublimate and Ehrlich's haematoxylin and eosin, 5 μ; x 975)

Figure 4. Ventral prostate of a male vole, obtained from the field in summer, showing columnar epithelium. (Formal sublimate and Ehrlich's haematoxylin and eosin, 5 μ; x 975)

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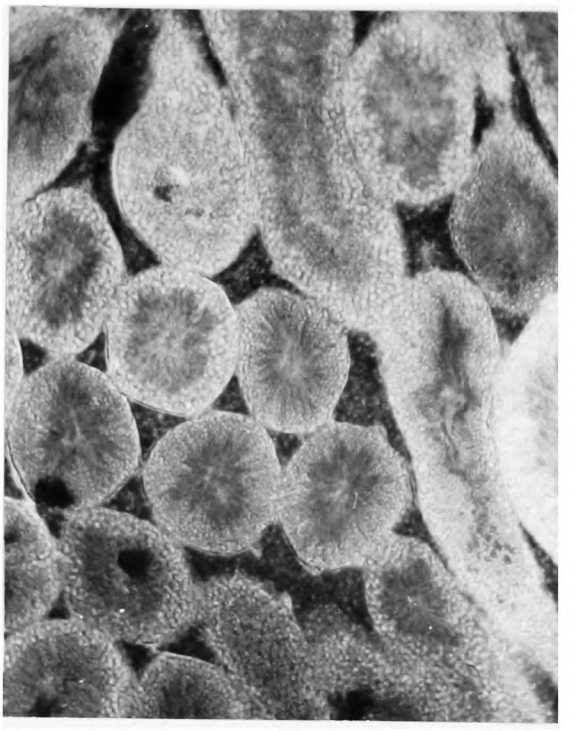

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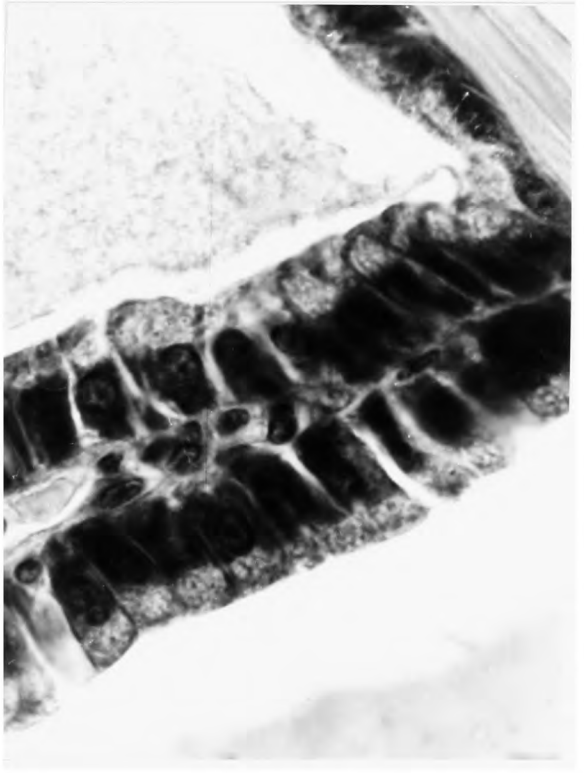

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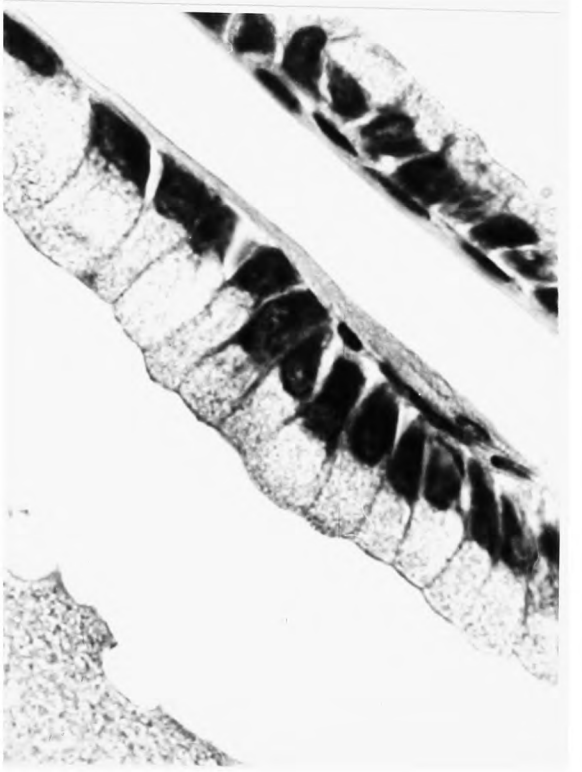


4.

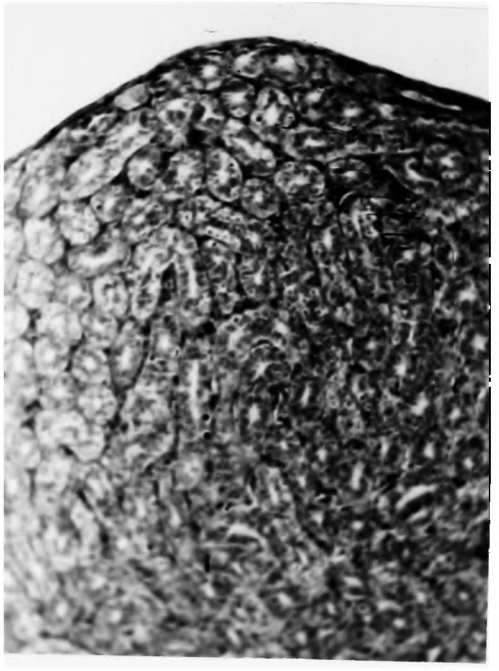
Plate II

Figure 1. Testis of an inhibited male vole, obtained from the field in winter, showing the thin tunica albuginea and small tubules, containing only spermatogonia and Sertoli cells. (Bouin and Ehrlich's haematoxylin and eosin, 5 μ; x 95)

Figure 2. Testis of a regressed male vole, obtained from the field in winter, showing the thick tunica albuginea, small tubules and lack of spermatogenic activity. (Bouin and Ehrlich's haematoxylin and eosin, 5 μ; x 95)

Figure 3. Testis of a regressed male vole, obtained from the field in winter, showing absence of lipid from scanty interstitial tissue. (Formal calcium and Sudan Black, 10 μ; x 95)

Figure 4. Ventral prostate of a male vole, obtained from the field in winter, showing high columnar epithelium. (Formal sublimate and Ehrlich's haematoxylin and eosin, 5 μ; x 975)



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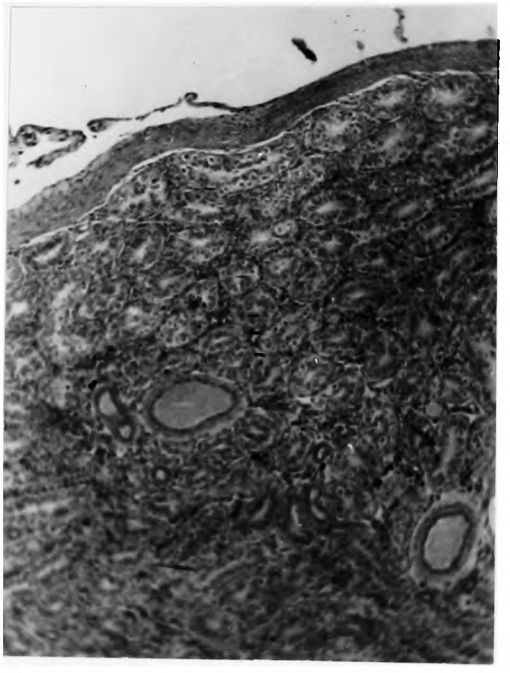

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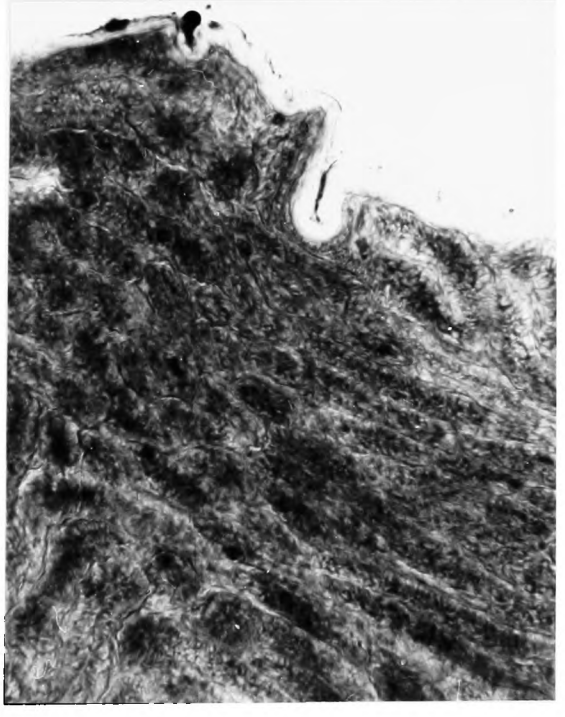

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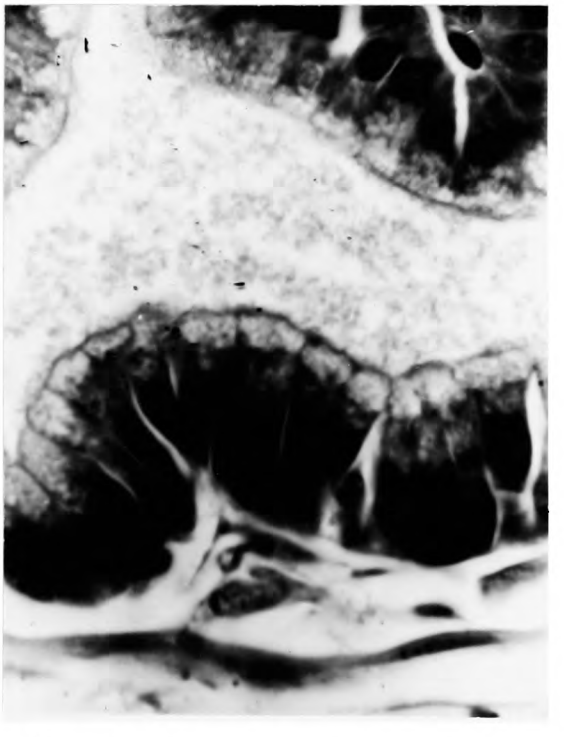

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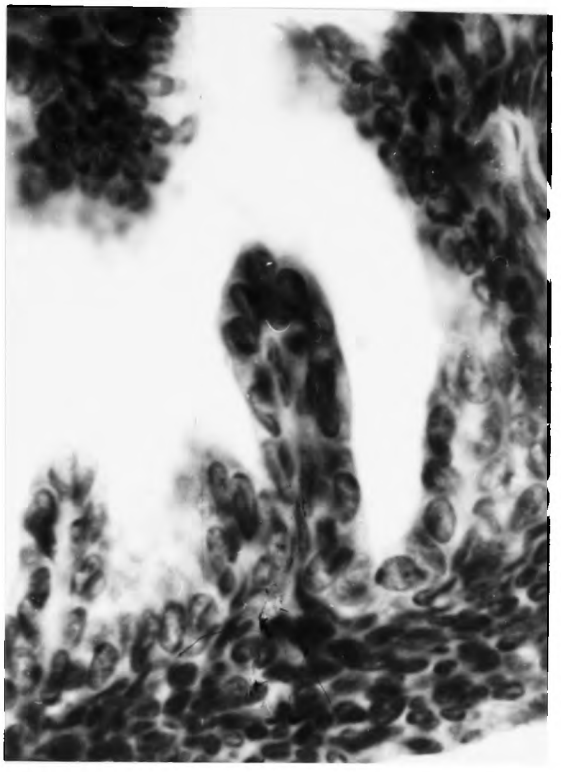
Plate III

Figure 1. Seminal vesicle of an inhibited male vole, obtained from the field in winter, showing low epithelium and relatively thin muscular wall (cp. Figure 2). (Formal sublimate and Ehrlich's haematoxylin and eosin, 5 μ; x 975)

Figure 2. Seminal vesicle of a regressed male vole, obtained from the field in winter, showing low epithelium and thick muscular wall. (Bouin and Ehrlich's haematoxylin and eosin, 5 μ; x 975)

Figure 3. Testis of a male vole, obtained from the field in the winter of 1958-59, showing sperm and quite well developed interstitial tissue. Testis weight 220 mg. (Formal sublimate and Ehrlich's haematoxylin and eosin, 5 μ; x 195)

Figure 4. Testis of a male vole, obtained from the field in the winter of 1958-59, showing spermatids, mitoses in the seminiferous epithelium and quite well developed interstitial tissue. Testis weight 30.1 mg. (Formal sublimate and Ehrlich's haematoxylin and eosin, 5 μ; x 195)



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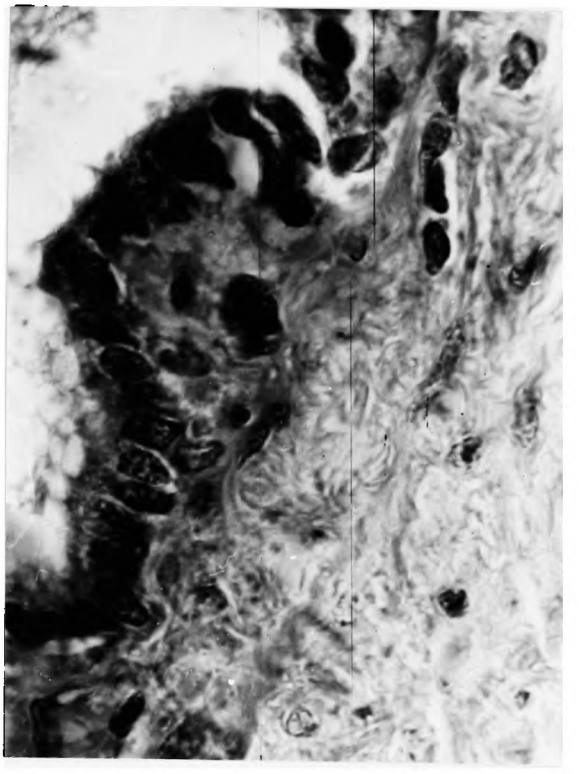

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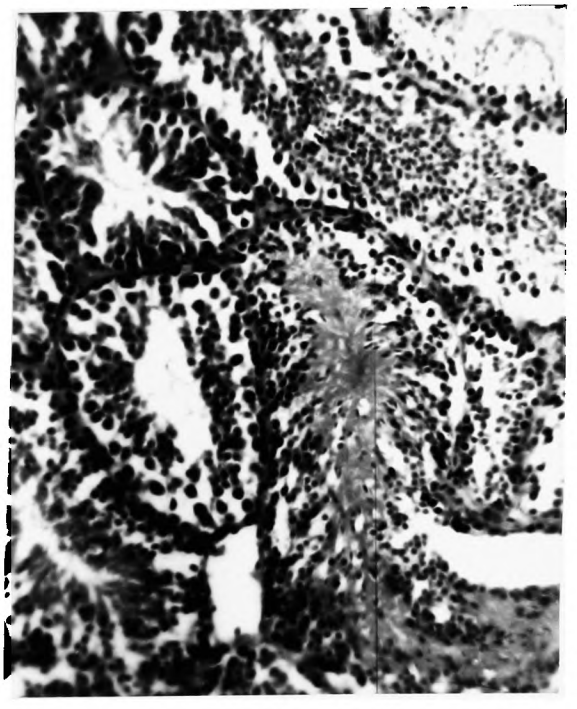

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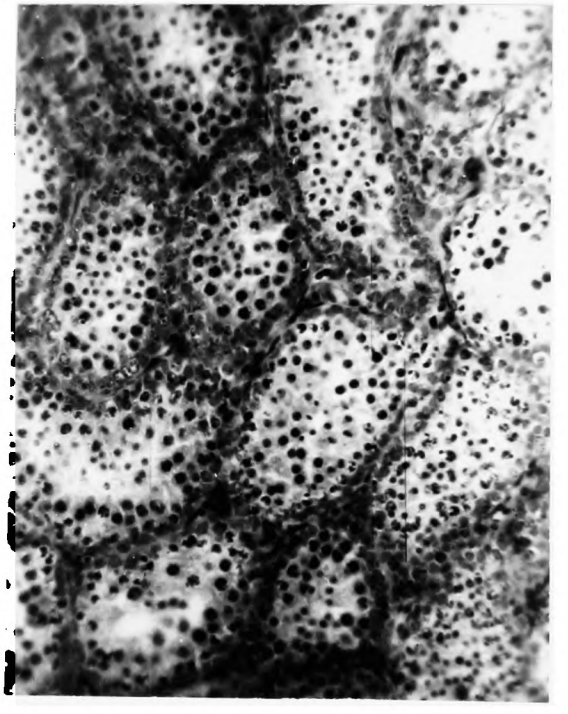

4.

Plate IV

Figure 1. Seminal vesicle of a male vole 7 days after castration to show reduction in epithelial height (cp. Plate I, Figure 3). (Formal sublimate and Ehrlich's haematoxylin and eosin, 5 μ; x 975)

Figure 2. Seminal vesicle of a male vole 112 days after castration to show low epithelium (cp. Plate III, Figure 2). (Formal sublimate and Ehrlich's haematoxylin and eosin, 5 μ; x 975)

Figure 3. Ventral prostate of a male vole 14 days after castration to show initial reduction in epithelial height (cp. Plate I, Figure 4). (Formal sublimate and Ehrlich's haematoxylin and eosin, 5 μ; x 975)

Figure 4. Ventral prostate of a male vole 112 days after castration to show high columnar epithelium (cp. Plate II, Figure 4). (Formal sublimate and Ehlich's haematoxylin and eosin, 5 μ; x 975)

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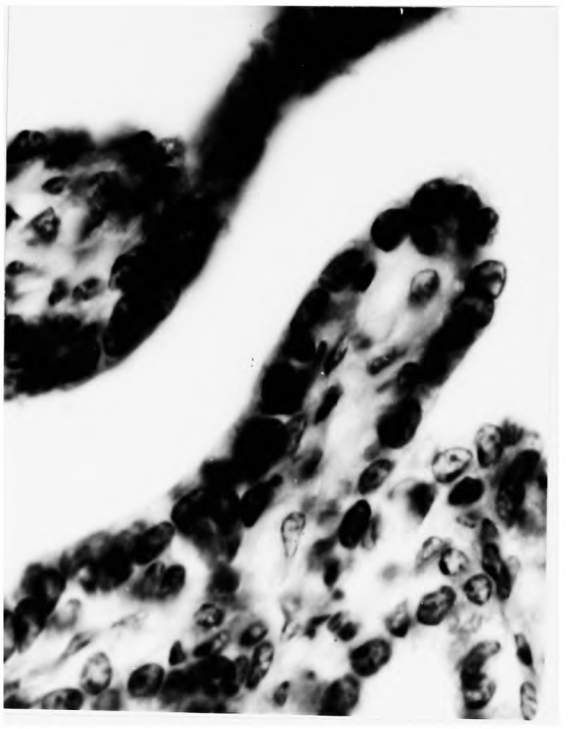

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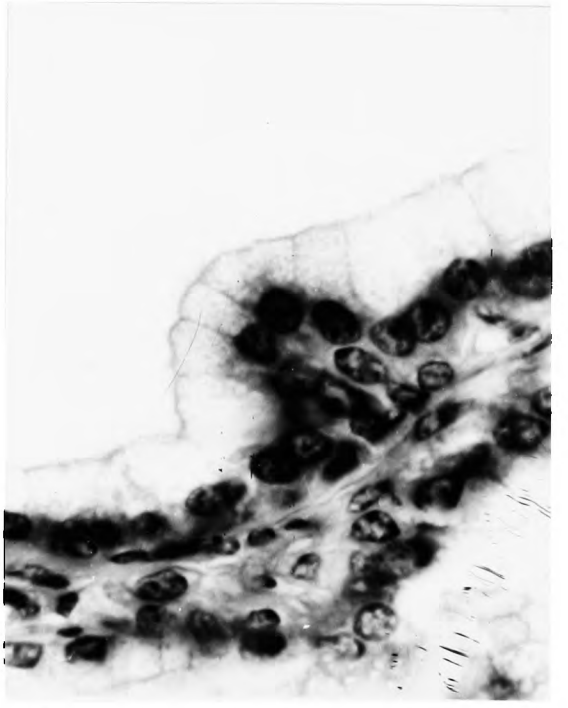


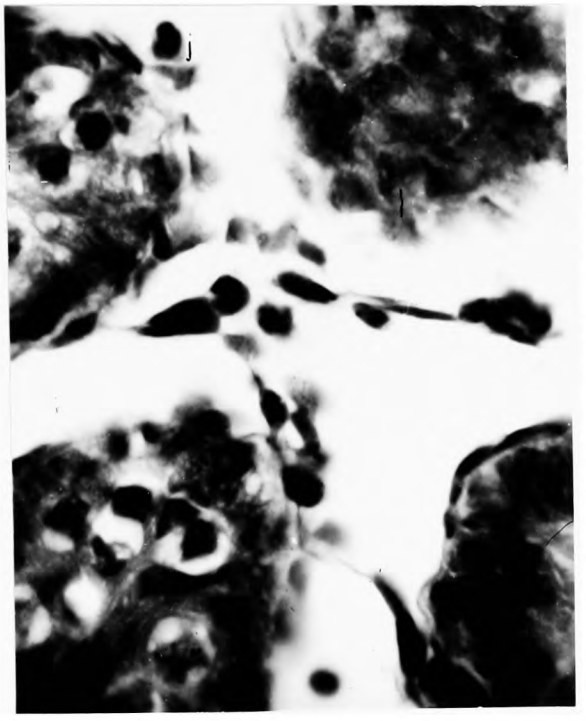
4.

Plate V. Response of sexually inhibited laboratory bred male voles to exogenous gonadotrophins. 5 μ sections of testes, fixed in Bouin's fixative and stained with Ehrlich's haematoxylin and eosin.

Figure 1. Control male to show interstitial tissue. Note pycnotic, mis-shapen nuclei and scanty cytoplasm. (x 975)

Figure 2. Treated male (low hormone dosage). The interstitial cells have vesicular nuclei and a moderate amount of cytoplasm. (x 975)

Figure 3. Treated male (high hormone dosage). The tubules are quite large and show all stages of spermatogenesis. Note looseness of seminiferous epithelium. (x 475)



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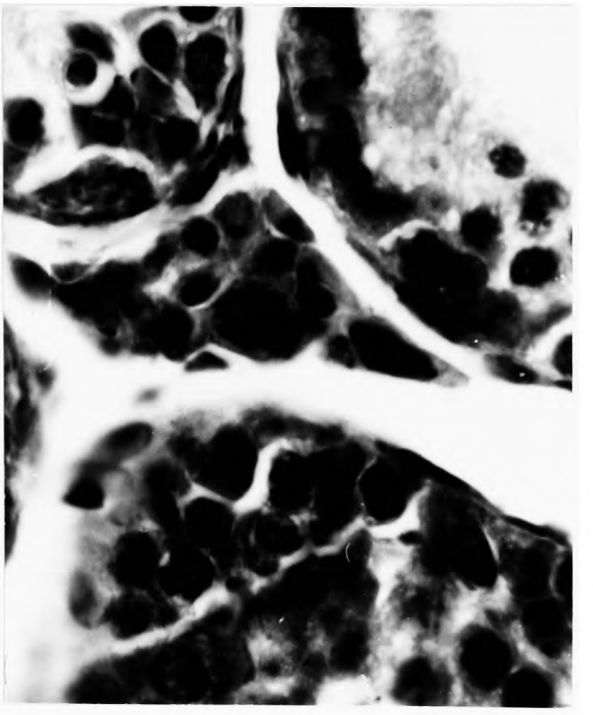

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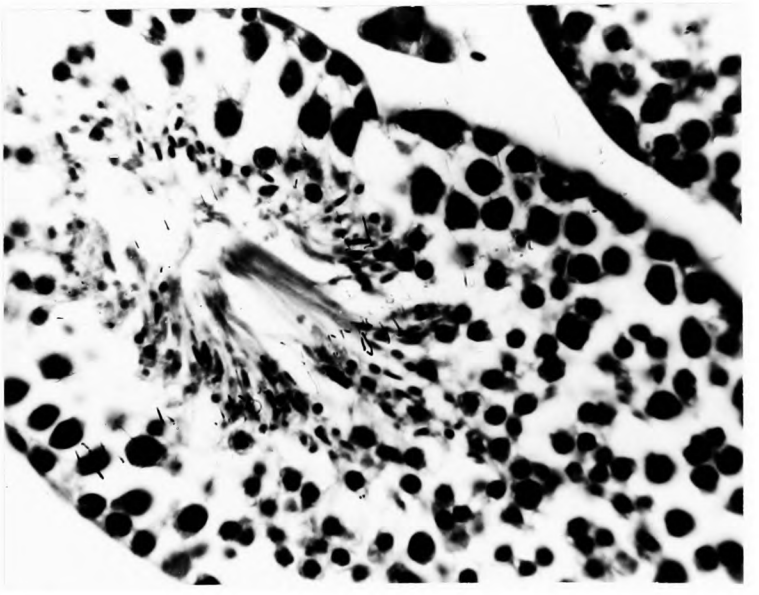


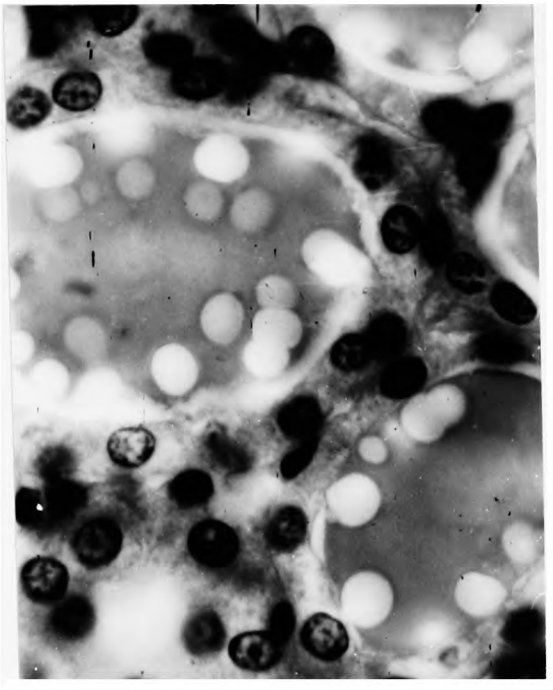
3.

Plate VI. Thyroid glands, fixed in Bouin's fixative and stained with Ehrlich's haematoxylin and eosin. 5 μ sections. All magnifications x 975.

Figure 1. Thyroid of a male vole, obtained from the field in summer, showing a moderate degree of activity. Cuboidal epithelium and colloid with vacuoles at its edge.

Figure 2. Thyroid of a female vole, obtained from the field in summer, showing an active thyroid. Low columnar epithelium and little colloid.

Figure 3. Thyroid of a female vole, obtained from the field in winter, showing little thyroid activity. Low epithelium and abundant non-vacuolated colloid.



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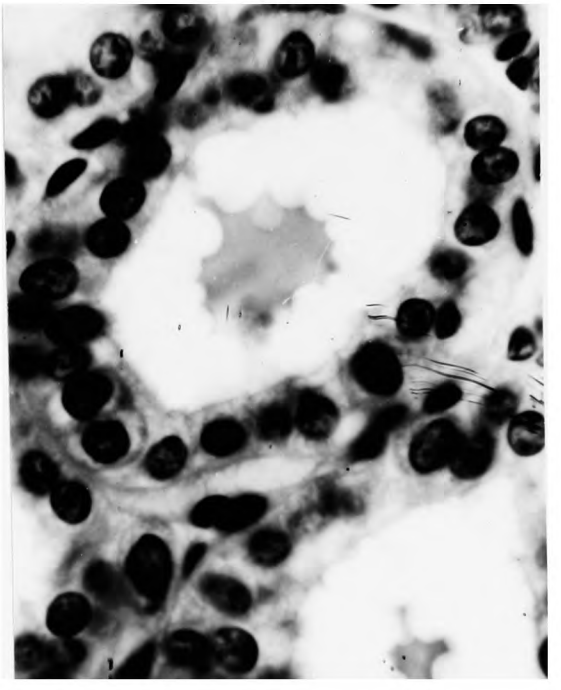

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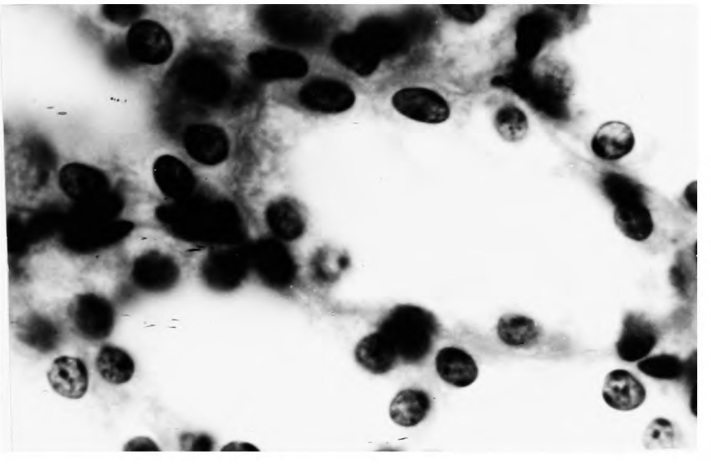


3.

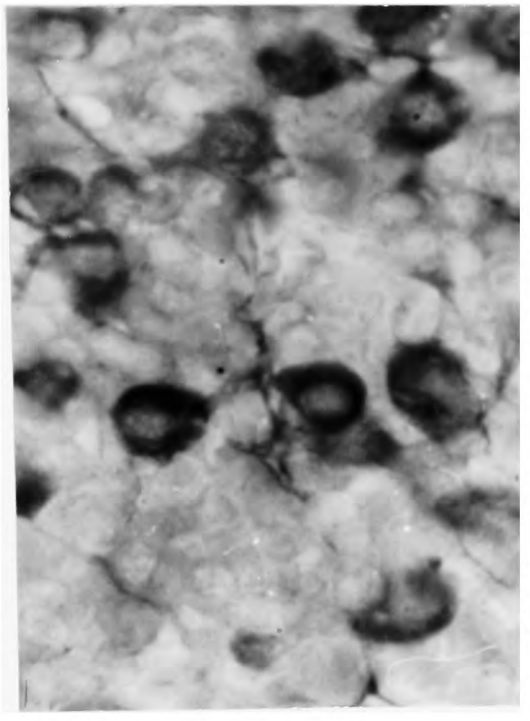
Plate VII

Figure 1. Round or oval gonadotrophs, strongly PAS positive, in the pituitary of a male vole, obtained from the field in summer. (Formal sublimate and PAS, 5 μ; x 975)

Figure 2. Angular thyrotrophs, strongly AF positive, in the pituitary of a male vole, obtained from the field in summer. (Formal sublimate and AF, 5 μ; x 975)

Figure 3. Two sorts of acidophil in the pituitary of a male vole, obtained from the field in summer: (1) Yellow cells which are homogeneous in appearance, round or oval with a round nucleus and with distinct cell boundaries. (2) Red cells with more or less oval nuclei, granular cytoplasm and indistinct cell boundaries. (Formal sublimate and PAS, Harris's haematoxylin and orange G, 5 μ; x 975)

Figure 4. Large oval cells (arrowed) in the pituitary of a laboratory bred male vole which had been castrated for 56 days. Note large granule in each large cell. Castration cells are also visible. (Formal sublimate and Mallory, 3 μ; x 975)



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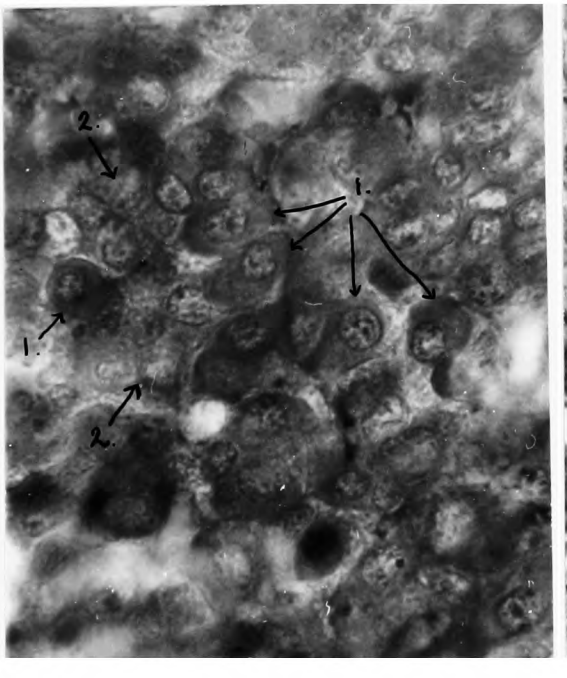

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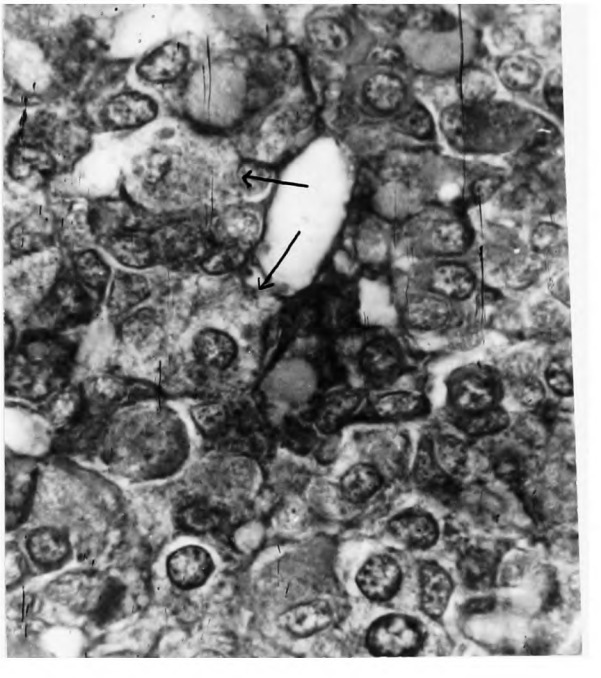


4.

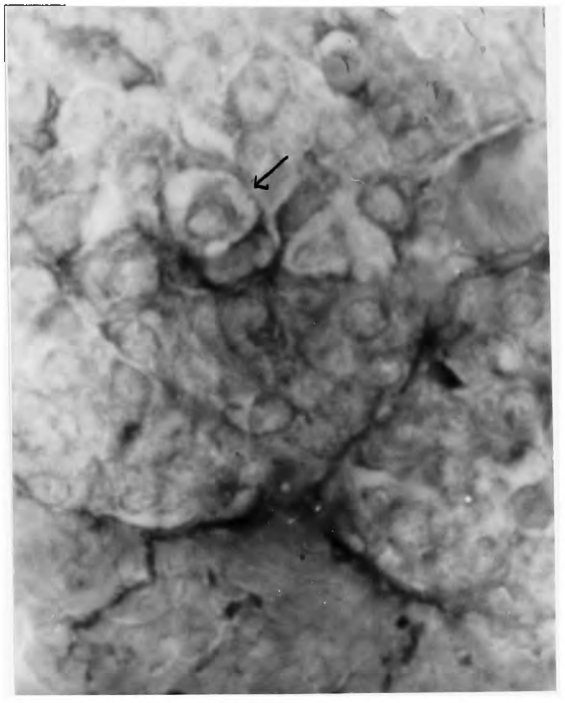
Plate VIII

Figure 1. Pituitary of a laboratory bred male vole, 7 days after castration, to show degranulation of gonadotrophs. One cell (arrowed) shows a few strands of PAS positive material. (Formal sublimate and PAS, 5 μ; x 975)

Figure 2. Pituitary of a laboratory bred male vole, 56 days after castration, to show colloid cells. One shows a single, the other three, colloid vesicles. (Formal sublimate and PAS, 5 μ; x 975)

Figure 3. Pituitary of a laboratory bred male vole, 84 days after castration, to show regranulation of gonadotrophs. Cell (arrowed) is small, strongly stained and contains a prominent negative image of the Golgi body. (Formal sublimate and PAS, 5 μ; x 975)

Figure 4. Pituitary of a male vole, obtained from the field in winter, showing vesiculated gonadotrophs. (Formal sublimate and PAS, 5 μ; x 975)



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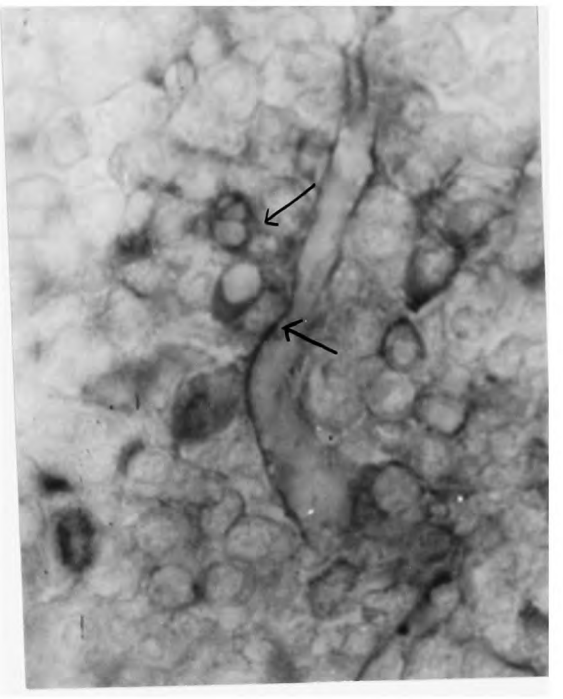

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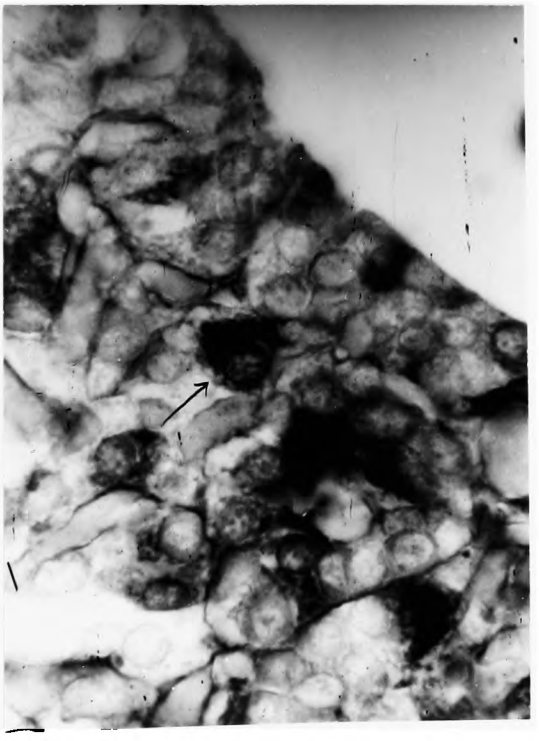

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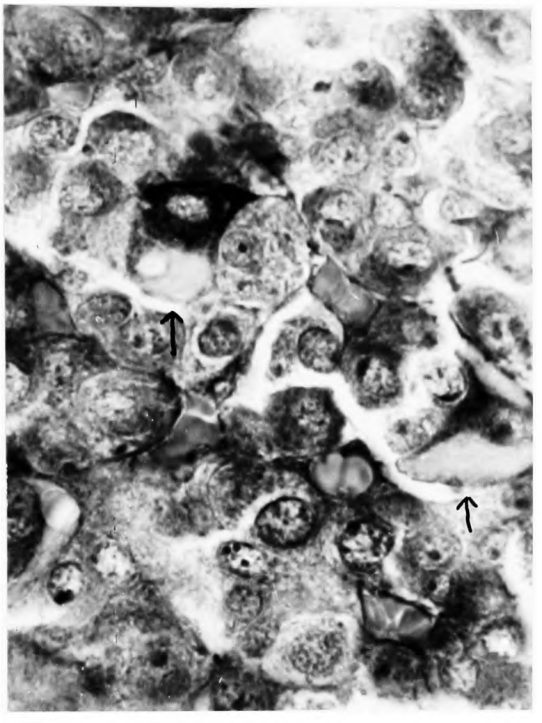
4.

Plate IX

Figure 1. Pituitary of a laboratory bred male vole, which had received 0.04% methylthiouracil for 2 days. To show stage in the establishment of thyroidectomy cells. The arrowed cells are angular in shape, contain AF positive granulation and also a patch of colloid material. (Formal sublimate and aldehyde fuchsin, Harris's haematoxylin and orange G, 5μ; x 975)

Figure 2. Pituitary of a laboratory bred male vole, which had received 0.04% methylthiouracil for 56 days. To show a fully developed thyroidectomy cell. The colloid contains an unstained vacuole. (Formal sublimate and aldehyde fuchsin, Harris's haematoxylin and orange G, 5 μ; x 975)

Figure 3. Pituitary of a laboratory bred virgin female vole which showed mammary development. The red cells (two of them have been indicated with arrows) show a large negative image of the Golgi body. (Formal sublimate and Mallory, 3 μ; x 975)



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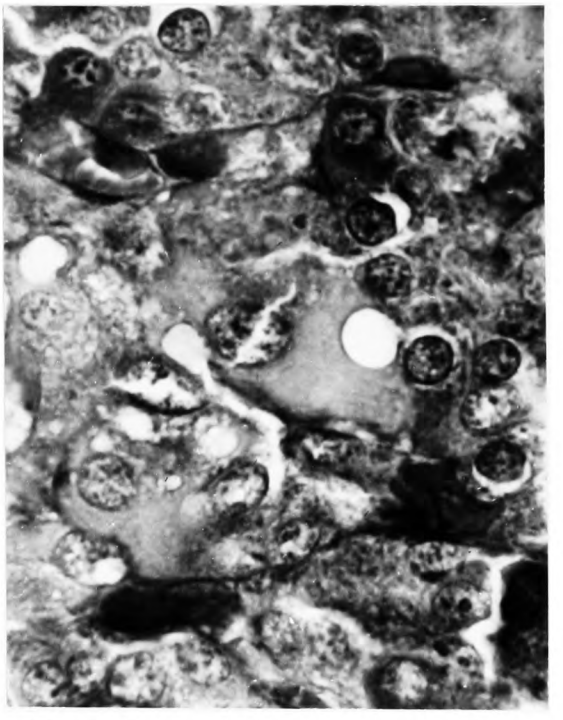

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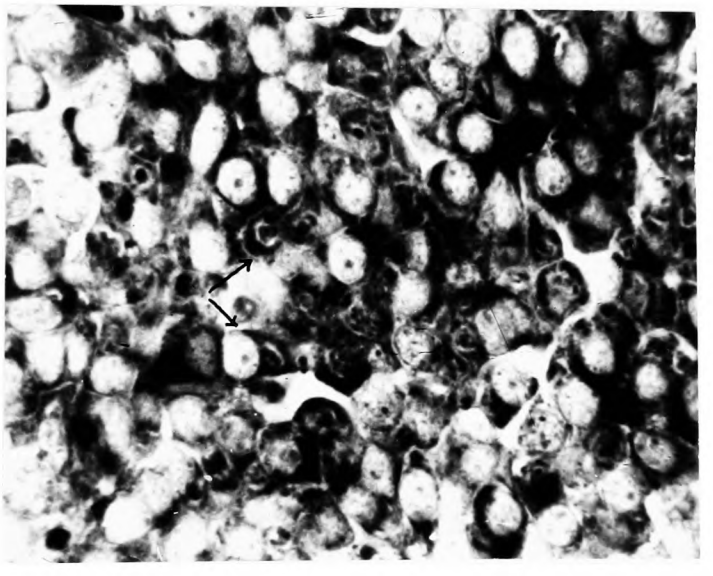


3.

Plate X

Figure 1. Adrenal of a male vole, obtained from the field in summer, to show slight X zone and well developed zona glomerulosa. (Formal sublimate and Ehrlich's haematoxylin and eosin, 5 μ; x 195)

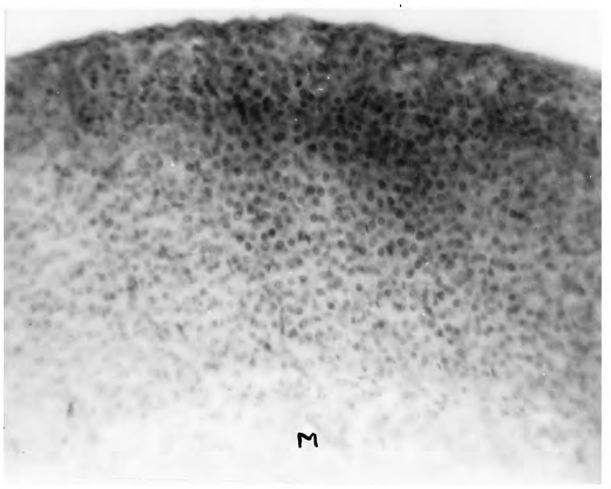
Figure 2. Adrenal of a male vole, obtained from the field in summer, to show absence of X zone and high concentration of lipids in the zona fasciculata. (Formal calcium and Sudan Black, 10 μ; x 195)

Figure 3. Adrenal of a male vole, obtained from the field in winter, to show X zone. (Formal sublimate and Ehrlich's haematoxylin and eosin, 5 μ; x 195)

Figure 4. Adrenal of a male vole, obtained from the field in winter, to show lipid droplets in the X zone and relatively few lipid droplets in the zona glomerulosa (cp. Figure 2). (Formal calcium and Sudan Black, 10 μ; x 195)

M = medulla

X



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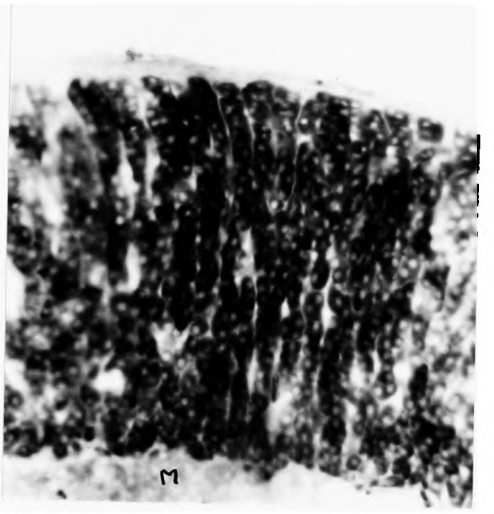

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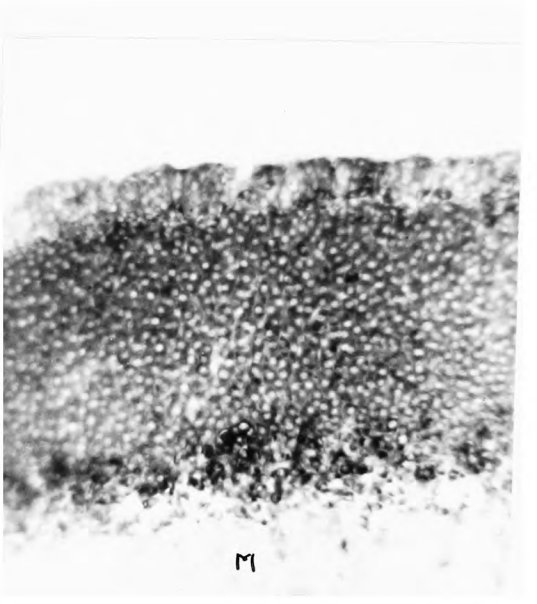

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Plate XI

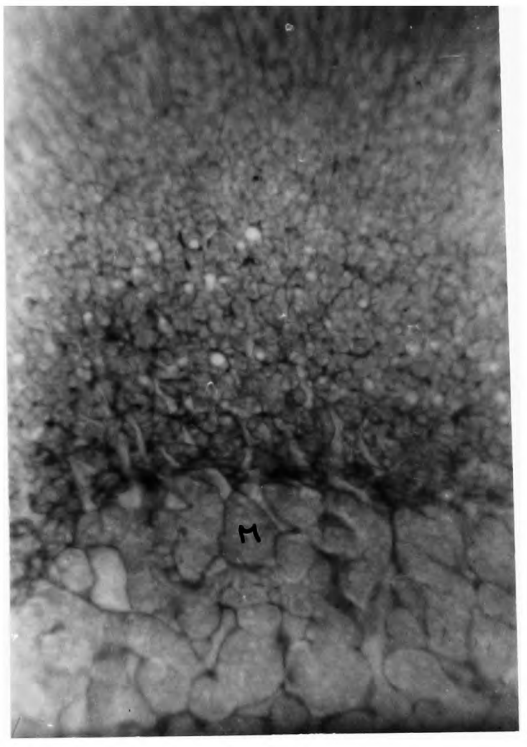
Figure 1. Adrenal of an inhibited winter male, to show vacuoles in the X zone and the presence of connective tissue fibres around the medulla. (Bouin and PAS, 5 μ; x 195)

Figure 2. Adrenal of a regressed winter male, to show the presence of connective tissue fibres around the medulla. (Bouin and PAS, 5 μ; x 195)

Figure 3. Adrenal of a laboratory bred male vole, 49 days after castration, to show the presence of an X zone. (Bouin and Ehrlich's haematoxylin and eosin, 5 μ; x 195)

Figure 4. Adrenal of a laboratory bred male vole, 84 days after castration, to show vacuoles in the X zone. (Formal sublimate and Ehrlich's haematoxylin and eosin, 5 μ; x 195)

M = medulla



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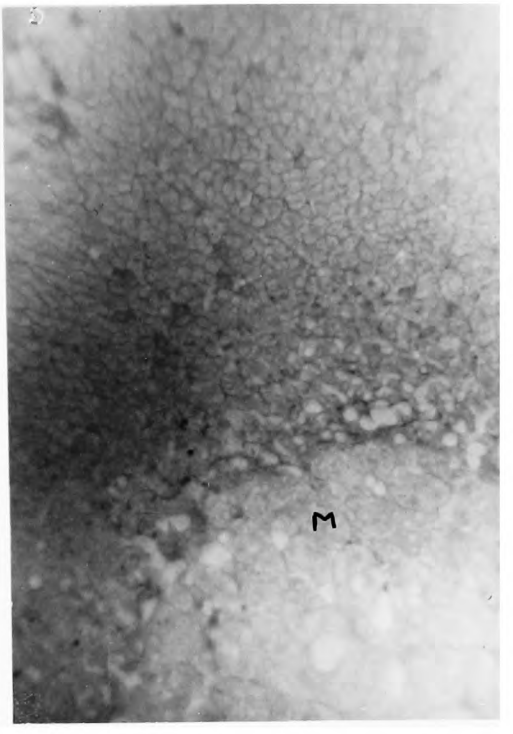

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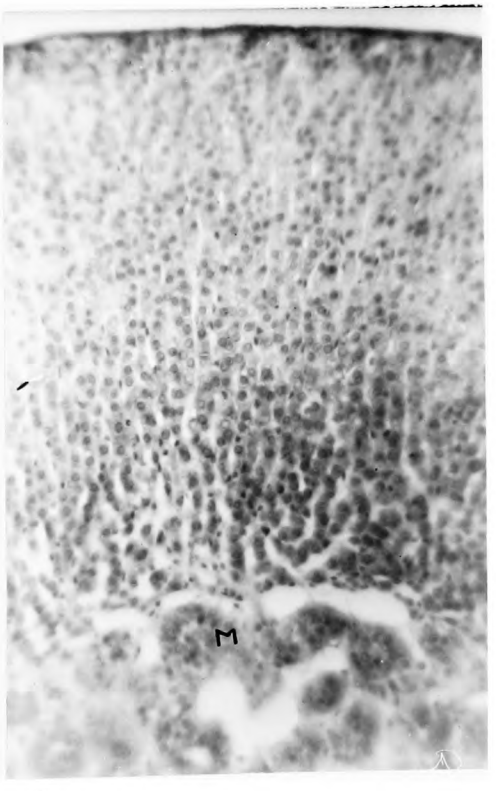

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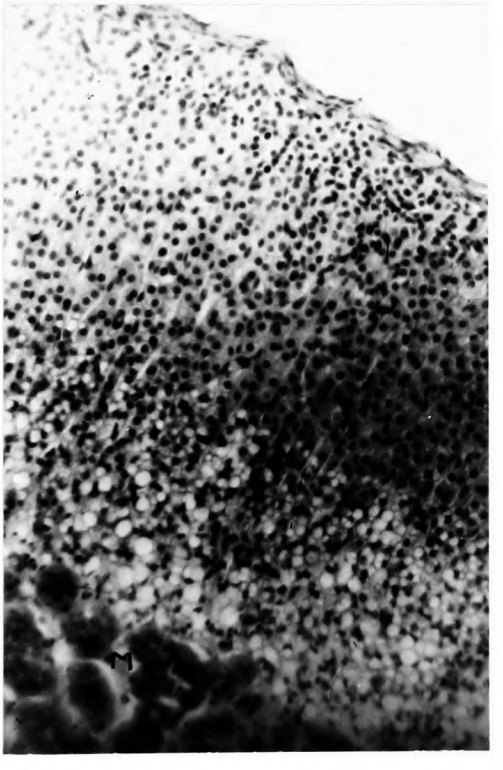


4.

Plate XII

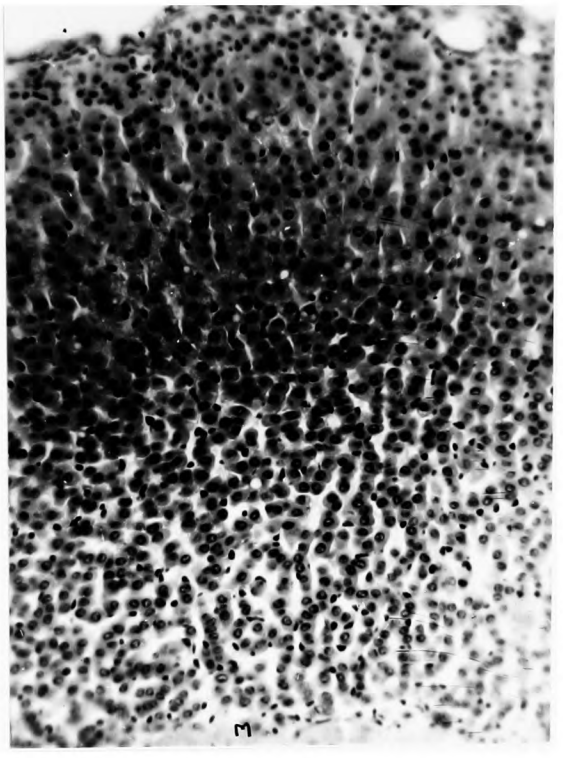
Figure 1. Adrenal of a primiparous female field vole, obtained from the field in summer, to show well developed X zone. (Formal sublimate and Ehrlich's haematoxylin and eosin, 5 μ; x 195)

Figure 2. Adrenal of a multiparous female field vole, obtained from the field in summer, to show well developed X zone. Note different structure of inner part of the zone, as compared with a primiparous female (Figure 1). (Formal sublimate and Ehrlich's haematoxylin and eosin, 5 μ; x 195)

Figure 3. As Figure 1; to show absence of connective tissue around the medulla. (Formal sublimate and PAS, 5 μ; x 195)

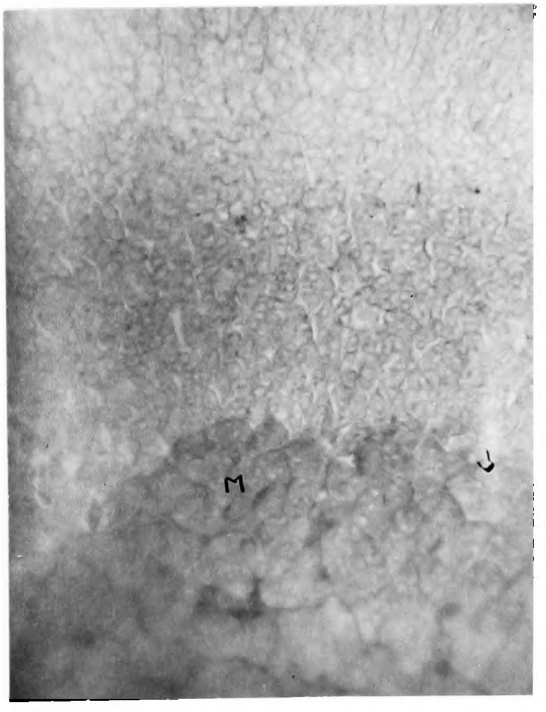
Figure 4. As Figure 2; to show presence of connective tissue around the medulla. (Formal sublimate and PAS; 5 μ; x 195)

M = medulla



1.

2.



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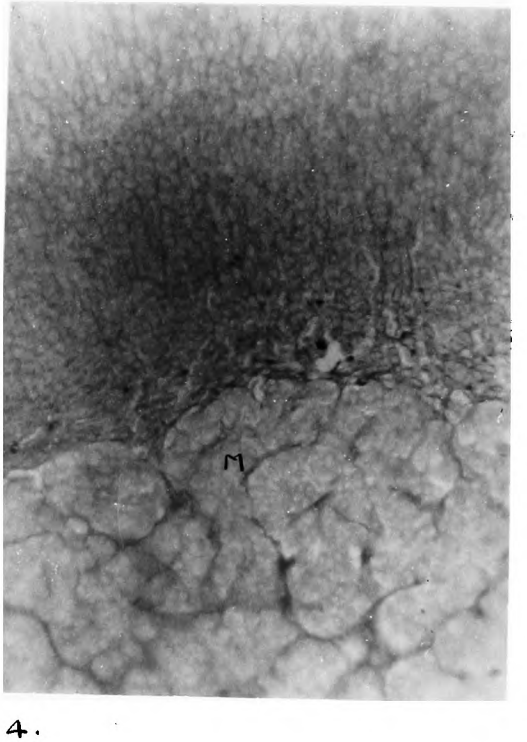

4.