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The Occurrence of *X*-zone in the Adrenal Glands of Two Rodent Species

[With 1 Table & Plates X—XII]

Examination was made of the occurrence of *X*-zone in the adrenal cortex of 228 mice — *Apodemus flavicollis* (M e l c h i o r, 1834) and 224 voles *Clethrionomys glareolus* (S c h r e b e r, 1780) caught at various times of the year, taking into account the sex and state of sexual activity of the animals. Differences were found to exist in the structure of *X*-zone in both species of rodents. In the case of *A. flavicollis* there are seasonal differences in the morphological structure of this stratum in sexually active males. *X*-zone does not differ from *zona reticularis* in very young males, but is very narrow in inactive individuals during winter or in a state of senile regression. *X*-zone disappears in the majority of pregnant females. In *C. glareolus* *X*-zone corresponds to *zona reticularis*, is subject to hypertrophy in pregnant females and disappears in sexually active males. The results obtained are compared with the corresponding relations found in laboratory mice.

I. INTRODUCTION

T a m u r a (1926) and M a s u i & T a m u r a (1926) described the juxtamedullary layer in the adrenal glands of mice, composed of cells with acidophilic cytoplasm with strongly basophilic nuclei. These authors considered this to be *zona reticularis*. H o w a r d (1927) termed this layer stratum *X*, later described as the androgenous, juxtamedullary, embryonic or sexual layer and as transitional cortex. For a long time stratum *X* was not distinguished from *zona reticularis*, but it is now known that these two zones differ from each other.

X-zone, characteristic of mouse adrenal glands, is dependent on age and sex. In unmated females it dissapears at different times, usually between the 32nd and 40th day of life (H o l m e s & D i c k s o n, 1971; C h e s t e r J o n e s, 1955, 1957; D e l o s t & C h i r v a n - N i a, 1958; S a k i z, 1959), and disappears during the first pregnancy, usually between the 7th and 15th day (C h e s t e r J o n e s, 1952). C h e s t e r

Jones (1949 b) suggests that *X*-zone may depend on gonadotropin, probably the luteinizing hormone (LH) which causes its maintenance. It disappears, however, after androgens have been administered (Deanesly & Parkers, 1937).

In males *X*-zone disappears with sexual maturation, probably as the result of secretion of testicular androgens (Howard, 1927; 1940; Chester Jones, 1955, 1957). Chester Jones (1952), in studies on transplantation of fertilized eggs to virgin mice and transplants of the prostate to the eye, concludes that ovarian androgens are secreted when *corpus luteum* is active, and that these androgens are responsible for the disappearance of *X*-zone during gestation.

There are few reports on *X*-zone in other animals, particularly in animals living in a wild state. The purpose of the present studies was to trace the behaviour of *X*-zone in two species of free-living rodents belonging to two different families: the yellow-necked field mouse

Table 1

Distribution of the material studied according to sex and degree of sexual activity.

Species	Males		Females				
	1	2	3	4	5	6	7
	Active	Non-active	Active	Non-active	Pregnant	Lactating	Lactating & pregnant
A. flavicollis	50	49	32	27	27	30	13
C. glareolus	48	53	10	61	33	20	17

belonging, like the white mouse, to the *Muridae* family and the red-backed vole belonging to the *Microtidae* family, and to compare it with *X*-zone in white mice.

II. MATERIAL AND METHODS

The studies were made on 228 individuals of the yellow-necked field mouse *Apodemus flavicollis* (Melchior, 1834) and 242 individuals of the bank vole *Clethrionomys glareolus* (Schreber, 1780), all of which were caught in the Białowieża National Park during the period from 1966 to 1969. Captures were made mainly in April, June and October, and once only in February and December.

The animals were divided into the 7 groups shown in Table 1, taking into account sex, sexual activity, gestation and lactation.

Sexual activity in males was determined on the basis of the macroscopic appearance of the gonads, the dimension of the testicles and their histological

picture. In females sexual activity was determined on the basis of the macroscopic appearance of the gonads, and gestation and lactation was also determined on the basis of macroscopic changes.

The animals were anaesthetized with ether, and the adrenal glands excised were fixed in Bouin's fluid and embedded in paraffin. Sections were stained with haematoxylin and eosine and by the Azan method.

III. RESULTS

1. *Apodemus flavicollis*

Group 1 — Active males

The adrenal cortex is composed of three layers: *zona glomerulosa*, *z. fasciculata* and *z. reticularis*. The reticular layer can be divided into two parts from the morphological aspect: outer and inner — juxtamedullar, that is, *X*-zone. The external part is composed of small cells with homogeneous cytoplasm, arranged in bands of cells. The bands form a network and there are numerous blood vessels between them. The inner part has a large number of fibrous elements, between which there are cells which change their appearance depending on the time of year or other factors. In February *X*-zone occupies approximately 1/2 the thickness of the whole *zona reticularis*. It is composed of numerous connective tissue fibres, between which there are cell elements, more numerous near the medulla. Small cells of irregular shape have only slightly acidophilic cytoplasm and cell nuclei staining fairly intensively. Between the cells numerous blood vessels occur which are compactly filled with erythrocytes (Fig. 1). In spring and summer, during the months of maximum reproduction, *X*-zone consists of large, single cells or cells occuring in agglomerations. Their cytoplasm is uniformly acidophilic. The spherical nuclei have a distinct chromatine stroma and a nucleolus. The cells of *X*-zone form a distinct band round the medulla and between them there are numerous connective tissue fibres (Fig. 2).

Stratum *X* in the adrenal glands of animals caught in October is composed of smaller cells (Fig. 3) than those in this layer obtained from animals caught in summer. The cytoplasm of the cells is neutrophilic, homogeneous, with usually large hyperchromatic nuclei. Numerous fibrous elements of connective tissue often round groups of cells and enlarged blood vessels occur between them.

Group 2 — Inactive males

In inactive males, as in the preceding group, X-zone forms the inner part of the *z. reticularis*. In these animals the cells of *X*-zone are small,

with weakly acidophilic cytoplasm and large nuclei with a distinct chromatinous stroma (Fig. 4). In very young males the *z. reticularis* was not found to be divided into an inner and outer part.

It is possible to distinguish in this group of animals males in which the testes have undergone regression. The dimensions of the testes are far smaller than in active individuals. Such animals are encountered in autumn and winter, and it may therefore be assumed that it is a case of seasonal or senile regression. *X*-zone in these males is narrow, consists of a small number of usually single cells with neutrophilic or basophilic foam-like cytoplasm (Fig. 5). The round or elliptic nuclei have one or two nucleoli and a distinct delicate chromatinous stroma.

Group 3 — Active females

While *X*-zone from the morphological aspect presented an almost uniform appearance in all individuals of active males during the period of full reproduction, in active females it is possible to distinguish three types of this layer.

Type I. In the adrenal glands of females it is possible to distinguish *z. reticularis,* fairly distinctly differentiated into an outer part, in which there are small cells with slightly acidophilic, or sometimes basophilic cytoplasm, and single blood vessels. *X*-zone, corresponding to the inner *z. reticularis,* is composed of fibrous elements, among which large cells with fairly homogeneous, slightly acidophilic cytoplasm, occur either singly or in scattered groups (Fig. 7). These cells have nuclei with a distinct chromatinous stroma.

Type II. *X*-zone does not occur at all in the adrenal glands of females (Fig. 8). Its place is taken by a band of fibres of connective tissue occurring on the boundary with the medullar part, and fine blood vessels.

Type III. Females of the type have a broad *X*-zone in the adrenals, composed of large cells with fairly homogeneous cytoplasm and large nuclei. Delicate fibres of connective tissue occur between the cells (Fig. 9).

Group 4 — Inactive females

The *z. reticularis* in inactive females is composed almost entirely of the juxtamedullar part, with *X*-zone. Bands of connective tissue can be seen, between which there are several layers of cells with light-coloured cytoplasm, with large nuclei and not numerous blood vessels (Fig. 6).

Group 5 — Pregnant females

The *zona reticularis* in these animals is fairly broad, and small cells with large nuclei occur in the external part. The inner part is composed of fibres of connective tissue and blood vessels. Cells of *X*-zone, with neutrophilic cytoplasm, occur between the fibres, either singly or in small numbers.

In single cases *X*-zone is composed of several rows of cells with fairly dense homogeneous cytoplasm with large nuclei. In these individuals homogeneous masses can be seen in some fields in which there are cell nuclei. It is probable that these are degenerate cells of this layer.

Group 6 — Lactating females

This group includes individuals in which there is no *X*-zone at all in the adrenals, individuals in which only single large cells of *X*-zone, with light coloured cytoplasm and large nuclei, are present in the adrenals (Fig. 11). In other cases *X*-zone is very broad, composed of large cells with light-coloured uniformly acidophilic cytoplasm (Fig. 12).

Group 7 — Pregnant lactating females

In pregnant females which are simultaneously lactating, as in the case of pregnant and lacting females, there are individuals which have a fairly broad *X*-zone and individuals in which this layer has disappeared, being replaced by numerous fibres of connective tissue with single small cells.

2. *Clethrionomys glareolus*

Group 1 — Active males

Active males have no *X*-zone at all. The cortex is composed of the *z. glomerulosa* and *z. fasciculata*, the latter extending to the medulla (Fig. 14). In some individuals accumulations of connective tissue and fairly numerous blood vessels occur round the medulla.

Group 2 and 4 — Inactive males and females

Inactive males and females of *C. glareolus* have been discussed jointly on account of the similarity of the morphological pictures.

The cortex consists of three layers: *z. glomerulosa, z. fasciculata* and *X*-zone, which is composed of cells with acidophilic, fairly homogeneous

cytoplasm. Scanty fibres of connective tissue and blood vessels occur between the cells (Fig. 13).

Group 3 — Active females

X-zone is fairly broad in this group of animals. Elements of connective tissue occupy the greater part of this layer. Cells with large nuclei and with uniformly staining cytoplasm occur between them. Near the medulla these cells occur only singly (Fig. 15).

Group 5, 6 and 7 — Pregnant, lactating and simultaneously pregnant and lactating females

The *X*-zone occurs in the adrenals of all these females. It is best developed in pregnant females (Fig. 16). In pregnant and simultaneously lactating individuals (Fig. 17) and in lactating females (Fig. 18) larger numbers of connective tissue fibres, between which there are fairly large cells with light-coloured cytoplasm, occur on the medullar side.

It will be seen from the results set out above that there are differences in the occurrence of *X*-zone in both the rodent species examined, and also in sexually active and inactive animals. A distinct seasonal cycle of changes in the morphological structure of *X*-zone can be seen in sexually active males of *A. flavicollis*, and the disappearance of this layer in pregnant females.

In *C. glareolus* the *z. reticularis* corresponds to *X*-zone, undergoes hypertrophy in pregnant individuals and disappears in sexualy active males.

IV. DISCUSSION

The endocrine function of the cells of *X*-zone of the adrenal cortex is a debatable question. The experiments made in this connection lead to the conclusion that the cells of this layer produce steroid sex hormones which are related to the sex glands (Jakowicki & Wiśniewski, 1962). Many atuhors have drawn attention to the fact that the occurrence of *X*-zone depends on the hormonal state of the animal's organism.

Initially secretion of gonadotropin by the pituitary gland is responsible for maintaining *X*-zone in the adrenal cortex of mice (Chester Jones, 1949 a, b; 1950), in whose opinion degeneration of *X*-zone in adult males, and in females pregnant for the first time, does not depend on the reduction or cessation of secretion of gonadotropin. In males disappearance of *X*-zone is caused by the direct action of androgens during the time when secretion of gonadotropin by the pituitary is

increased (Chester Jones, 1949 a). In female mice also X-zone disappears during the first pregnancy, during which time there is usually considerable secretion of gonadotropin by the pituitary gland.

Holmes & Dickson (1971) found that when testosteron is injected into 21-day old virgin female mice this affects X-zone and causes it to disappear completely within 66 hours from the first injection.

The effect of endogenous androgens was investigated earlier by many authors, who suggested that these hormones cause disappearance of X-zone in adult males (Chester Jones, 1957, gradual disappearance in sexually mature but virgin females and rapid disappearance in females pregnant for the first time (Chester Jones, 1952, 1955; Deanesly, 1958; Kirby, 1966). An experiment was carried out to provide support for the above suggestions, and the result showed that androgens are present in females and also that the ovary is able to convert progesterone into androgens (Vinson & Chester Jones, 1963; Lloyd, 1966; Aakvaag, 1969).

X-zone in sexually active males of *A. flavicollis* exhibits seasonal differences from the morphological aspect. During winter and autumn it is composed of small cells, whereas in spring and summer the cells of this layer are large and form a broad band round the medulla. The presence of X-zone and enlargement of its cells during the period when reproduction intensifies may be connected with the males' greater need for androgenous hormones during this period. Grounds for reaching this conclusion are provided by the fact that X-zone disappears in males with regression of testes.

The function of the pituitary gland in free-living animals decreases in winter, whereas in summer there is considerable intensification of its activity (Rigaudiere, 1969). As the activity of hypophysis during the winter period decreases it is probable that the secretion of *LH* which is responsible for maintaining the function of X-zone in mice becomes lower (Chester Jones, 1957). At this time the X-zone of the active males adrenals of *A. flavicollis* is built of cells which give an impression of inactive ones.

Disappearance of stratum X in females of *A. flavicollis* in probably connected with pregnancy having taken place, as is the case with white mice (Chester Jones, 1957). It is, however, difficult to understand why X-zone is present in lactating females and in females simultaneously pregnant and lactating.

The X-zone is also present in pregnant, lactating and simultaneously pregnant and lactating females of *C. glareolus* (*cf.* also Delost & Delost, 1954). In the males of these animals, as is the case with white

mice, *X*-zone occurs in immature individuals, but disappears when they attain sexual maturity.

The studies made show that there are differences in the occurrence of *X*-zone in the two species of free-living animals discussed in this study, and that there are also differences in its disappearance. It must be assumed that the mechanisms of both the maintenance and disappearance of this layer differ in different species of animals and cannot be directly compared with observations made on laboratory animals.

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WYSTĘPOWANIE WARSTWY *X* W NADNERCZACH *APODEMUS FLAVICOLLIS* (MELCHIOR) I *CLETHRIONOMYS GLAREOLUS* (SCHREBER)

Streszczenie

Przeprowadzono badania nad występowaniem warstwy *X* kory nadnerczy u 228 osobnikach myszy wielkookiej leśnej, *Apodemus flavicollis* (Melchior, 1834) i 242 osobnikach nornicy rudej, *Clethrionomys glareolus* (Schreber, 1780). Zwierzęta pochodziły z Białowieskiego Parku Narodowego a łowione były w latach od 1966 do 1969. Wyłowy odbywały się w kwietniu, czerwcu, październiku, grudniu i lutym.

Zwierzęta podzielono na 7 grup uwzględniając płeć, aktywność płciową, ciążę i karmienie. Samce podzielono na: aktywne płciowo i nieaktywne płciowo, samice podzielono na: aktywne i nieaktywne płciowo, ciężarne, karmiące oraz ciężarne karmiące.

Nadnercza utrwalano w płynie Bouina i zatapiano w parafinie. Skrawki barwiono hematoksyliną i eozyną oraz metodą Azan.

Z przeprowadzonych badań wynika, że istnieją różnice w występowaniu warstwy *X* nadnerczy u *Apodemus flavicollis* i *Clethrionomys glareolus*. Występują różnice w budowie morfologicznej warstwy *X* nadnerczy u samców aktywnych i nieaktywnych płciowo myszy leśnej, jak również różnice w budowie morfologicznej tej warstwy u samców aktywnych myszy leśnej, w cyklu rocznym.

EXPLANATION TO PLATES X—XII

Plate X.

Fig. 1. The adrenals of a male yellow-necked field mouse, sexually active, in winter. *X*-zone is formed of small cells located between numerous blood vessels Magn. 160 × [1]).

Fig. 2. *X*-zone of the adrenals gland in a sexually active male in spring. It is formed of large cells arranged in a wide band round the medulla. Magn. 320×.

Fig. 3. Adrenals of a sexually active male yellow-necked field mouse in October *X*-zone is formed of small cells. Magn. 240×.

Fig. 4. Adrenals of a sexually inactive male yellow-necked field mouse. Groups of small cells belonging to *X*-zone can be seen. Magn. 160 ×.

Fig. 5. Adrenals of a male field mouse with regression of the testes. *X*-zone occurs in the form of single cells near the medulla. Magn. 320 ×.

Fig. 6. Adrenals of female field mouse, not sexually active. *X*-zone, formed of small cells arranged in two or three layers can be seen round the medulla. Magn. 320 ×.

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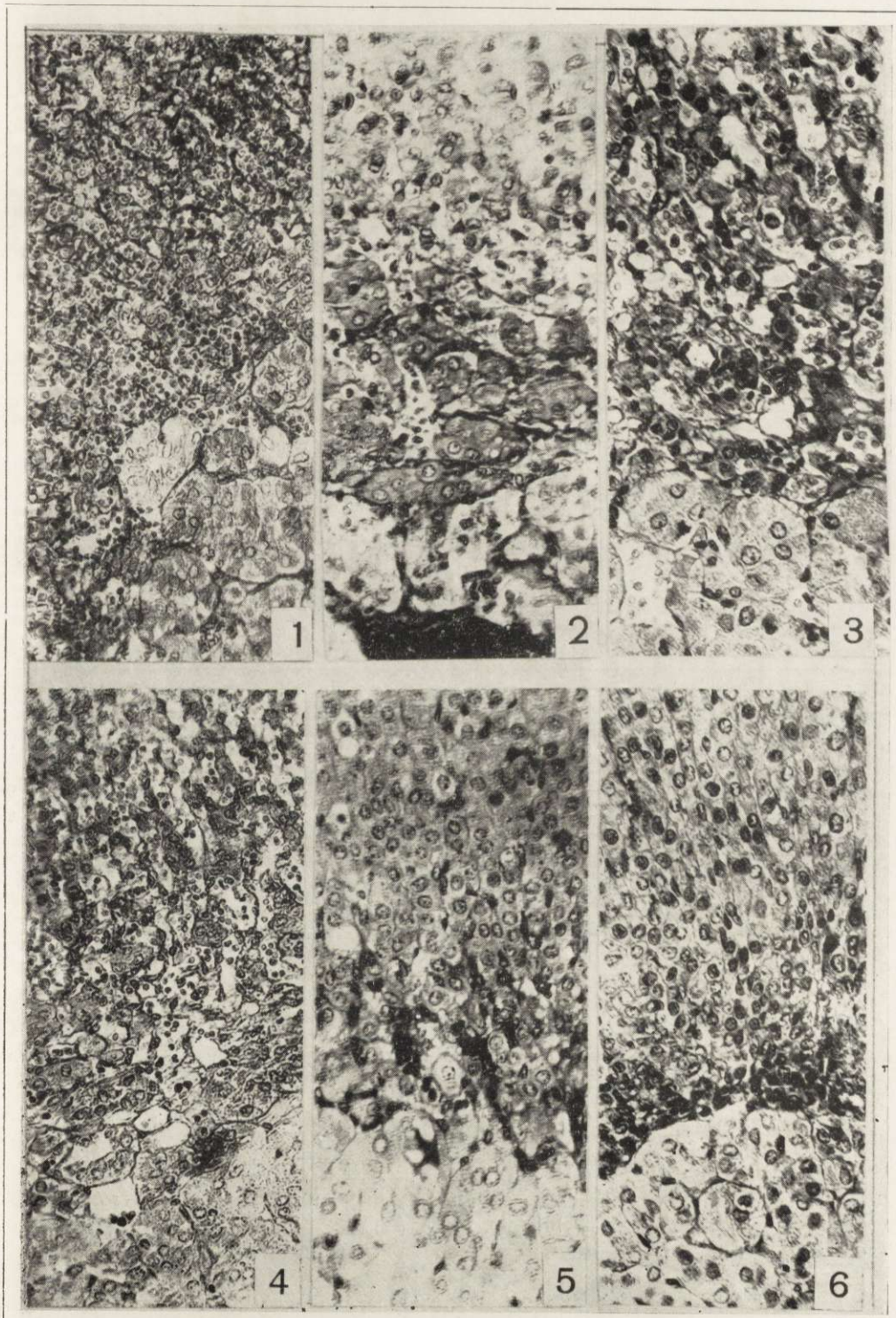
[1]) All the microphotographs were made of sections stained by the Azan method.



S. Siuda *auctor phot.*

Plate XI.

Fig. 7. Adrenals of type I sexually active female field mouse. *X*-zone consists of fairly large cells arranged in two or three layers. Magn. 160 ×.

Fig. 8. Adrenals of type II sexually active female field mouse. *X*-zone cannot be seen in the cortex. Magn. 240 ×.

Fig. 9. Adrenals of type III sexually active female field mouse. *X*-zone consists of large cells forming a broad band round the medulla. Magn. 320 ×.

Fig. 10. Adrenals of a lactating female field mouse. Single connective tissue fibres occur in place of *X*-zone. Magn. 240 ×.

Fig. 11. Adrenals of lactating female field mouse. Single cells of *X*-zone can be seen. Magn. 240 ×.

Fig. 12. Adrenals of lactating female field mouse. *X*-zone is very broad, and consists of large cells arranged in groups. Magn. 240 ×.

Plate XII.

Fig. 13. Adrenals of sexually inactive male bank vole. *X*-zone occurs in the form of a broad band of cell elements and vessels staining a darker colour. Magn. 320 ×.

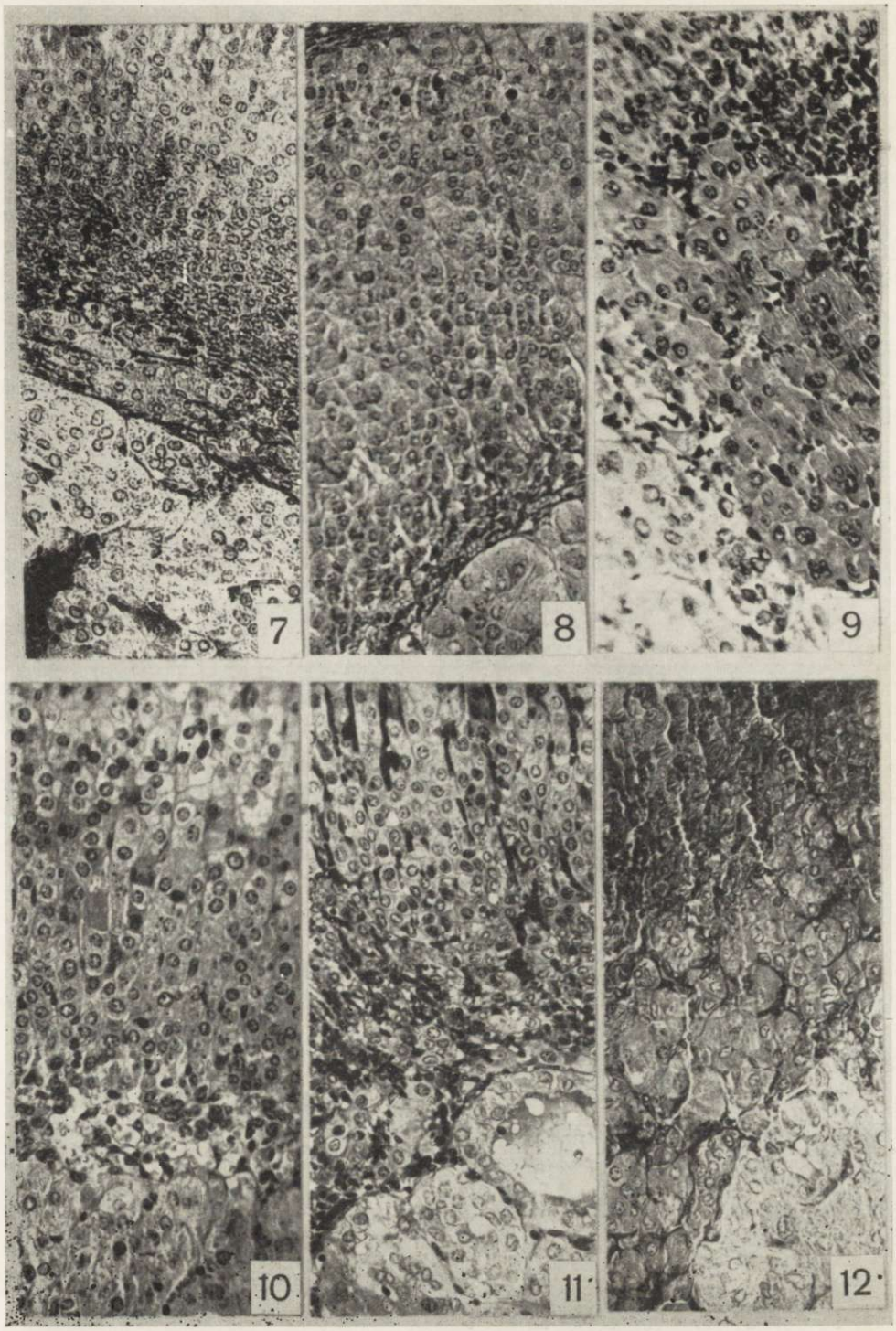
Fig. 14. Adrenals of sexually active male bank vole. There is no *X*-zone and the *zona fasciculata* extends to the medullar part. Magn. 320 ×.

Fig. 15. *X*-zone of the adrenals in a sexually active female bank vole is formed of single cells, while a broad band of connective tissue fibres occurs near the medulla. Magn. 320 ×.

Fig. 16. *X*-zone of the adrenal in a pregnant female bank vole is formed of several layers of cells. Magn. 320 ×.

Fig. 17. The adrenals of a pregnant and simultaneously lactating female bank vole. Connective tissue fibres appear on the medullar side. Magn. 320×.

Fig. 18. The adrenals of a lactating female bank vole. *X*-zone is relatively well retained, scanty connective tissue fibres and numerous blood vessels can be seen. Magn. 320 ×.



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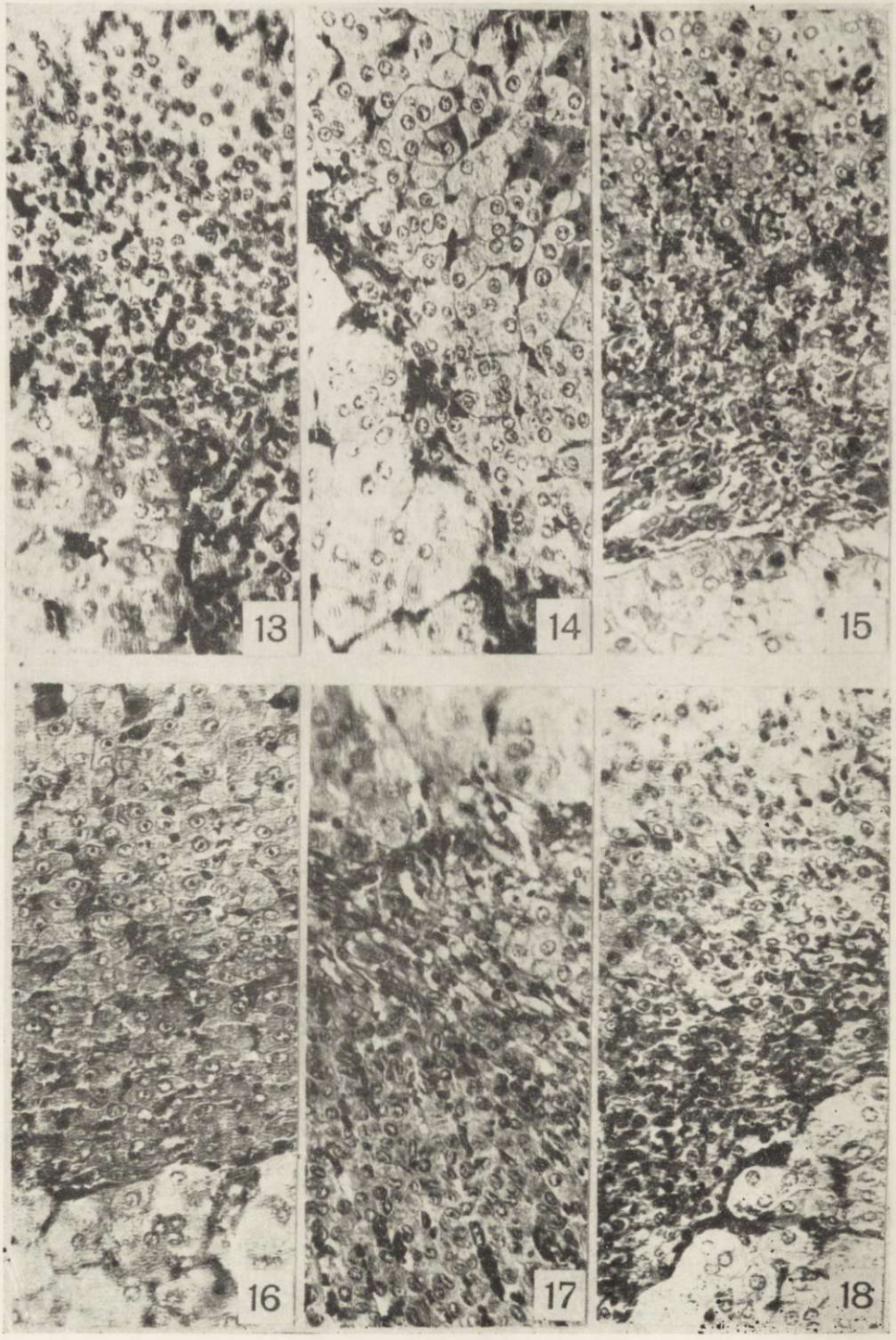

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