# A C T A T H E R I O L O G I C A

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# Microscopic Structure of the Thyroid Gland in *Apodemus flavicollis* (Melchior, 1834) Over the Yearly Cycle

#### [With 1 Table & Plates VII-IX]

Examination was made of the thyroid gland of 265 yellow-necked field mice obtained by trapping at different times of the year in the Białowieża National Park, during the period from 1966—1969, taking into account the sex, age and state of sexual activity of the animals. At the age of approximately 4 weeks, irrespective of sex and season, the histological structure of the thyroid gland was found to be uniform and correspond to intensified endocrinal activity. At the age of 2 months to one year marked seasonal variations occurred in the structure of this gland, while involutional changes were observed in the thyroid gland, of animals over 1 year old. Similar changes occur sporadically also in animals less than 1 year old, but only during the winter period. Gestation and lactation almost always exerted a stimulating effect on the function of the thyroid gland, and individual differences were also observed in the morphological pictures of the thyroid gland in animals belonging to uniform groups.

#### I. INTRODUCTION

Histomorphological changes in the thyroid gland of small mammals over the yearly life cycle have only sporadically been described (D elost & Naudy, 1956). We have not encountered a study of this kind referring to the yellow-necked field mouse, *Apodemus flavicollis* (Melchior, 1834) in the literature at our disposal. It therefore appeared useful to trace the histological changes in the thyroid gland of this rodent due to seasonal variations over the yearly cycle, simultaneously taking into account the influence of age, sex and state of sexual activity and comparing the degree to which these changes occur in successive study years (1966—1969).

# II. MATERIAL AND METHODS

A total number of 265 yellow-necked field mice were caught in the Białowieża National Park. Detailed data on the time of capture, and

number and sex of the animals examined, are given in table 1. The animals were killed under ether anaesthesis, then both lobes of the thyroid gland were excised, one of which was always fixed in Bouin's fluid, and the other in one of the three following fixing fluids: Carnoy's, Hell's or Lison-Vokaer. Paraffin preparations sectioned to  $6 \mu$  were stained with haematoxylin-eosin, gallocyanin and by the Azan and PAS + ferrocolloid methods. The relative age of the animals was defined on the basis of tooth wear (A d a m c z e w s k a - A n d r z e j e w s k a, 1967). Five age groups were distinguished: group I — animals about 4 weeks old, group II — animals about 2 months old, III — animals about 5 months old, IV — about 9 months old and V — animals over 1 year old.

# Table 1

List	of	numbers	of	Apodemus	flavicollis	caught	in		
		different years.							

	Males	Females	Total
April, 1966	4	7	11
July, 1966	7	17	24
October, 1966	21	23	44
December, 1966	5	1	6
February, 1967	7	2	9
April, 1967	7	7	14
June, 1967	14	11	25
October, 1967	12	13	25
April, 1968	5	3	8
October, 1968	15	17	32
June, 1969	20	25	45
October, 1969	13	10	23
1966—1969	129	136	265

Sex and sexual activity were defined on the basis of macroscopic inspection of the internal genital organs after opening the abdominal cavity, and in the case of males the weight of the testes were also taken into consideration. Gestation and lactation were ascertained in females on the basis of macroscopic changes. The maximum dimensions of each thyroid gland visible in serial sections, were used in the studies. At the same time measurement was made of the size of vesicles, height of the epithelium lining the glands and the diameter of its cell nuclei. Vesicles were defined as: small — with diameter not exceeding 25  $\mu$ , medium — from 25 to 50  $\mu$ , large from 50 to 100  $\mu$ , very large, with the greatest diameters, usually exceeding 100  $\mu$ . The blood supply, amount of connective tissue between vesicles, density of the colloid contained in the vesicles and intensity of staining of the cytoplasm in the vesicular cells were all

included in the description. We also took into account any changes in the structure of the gland, such as, *e.g.*, foci of the »necrotic type«, infiltrations from the pseudolymphocyte cells and proliferation of yellow or brown adipose tissue in the stroma. The assumed degree of secretory function was defined on the basis of observations of all the above morphological characters of the gland.

#### III. RESULTS

# 1. Animals from Winter Trapping

Examination was made of the thyroid in 6 field mice caught in December 1966. No differences connected with sex were found in its histological structure. The typical characters during this period were the small dimensions of the thyroid and slight individual changes in microscopic structures. Medium vesicles were observed greatly to predominate over the somewhat small number of large vesicles and the very infrequently occurring very large vesicles. The last two types of vesicles were located peripherally in the gland. The vesicles were lined with simple cubical epithelium (Fig. 1 and 2, Plate VII), but with markedly flat epithelium in the large and very large vesicles. Its cells contained finegrained slightly acidophil cytoplasm with round, or occasionally oval, small nuclei, staining a dark colour with haematoxylin. The vesicles were usually filled with dense colloid, which was strongly basophil in the large and very large vesicles, but usually acidophilic in the medium and small vesicles. Colloid was observed to stain many colours in some of the vesicles. In addition it was found that the blood supply to the thyroid is very poor and that there is abundant growth from the periphery (particularly in the region of penetration of blood vessels) of connective tissue containing numerous brown fat cells (Fig. 1, Plate VII).

During the second winter season (February 1967) the thyroid was taken from 9 animals. Larger general dimensions of the gland were found than in animals from December 1966, particularly in sexually active individuals. In addition the vesicles on the periphery of the gland were larger. The most important changes, however, were those in the appearance of the vesicular epithelium, which was higher in sexually active males than in females. Changes in the height of cells lining single vesicles were even frequently observed. Cells in the places in which columnar epithelium occurred had l'ght-coloured cytoplasm and relatively large, round nuclei. Intensive vacuolization of colloid was simultaneously observed near the columnar epithelium, and it was also observed to become distinctly less dense on the margins, while the capillary vessels were enlarged. The colloid located more internally in the vesicles

was usually dense (Fig. 3, Plate VII). The changes described above often occupied the external parts of the thyroid and applied both to small and large vesicles, and marked individual variation can be observed in this respect. In sexually active females (Fig. 4, Plate VII) the vesicular epithelium was more uniform in height.

# 2. Animals from Spring Trapping

Examination was made of the thyroid in 33 yellow-necked field mice (April 1966—68). Intensification was found of the fluctuations, already observed in February, in the height of the vesicular epithelium, with more frequent occurrence of columnar epithelium than before. This is most clearly seen in sexually active males, particularly in material from 1966 (Fig. 5 and 6, Plate VII) and least distinctly in material from 1967 (Fig. 7, Plate VIII). At the same time differences connected with sex in the structure of the thyroid were faintest. The greatest uniformity in thyroid structure in different individuals was observed in sexually active males caught in April 1966.

# 3. Animals from Summer Trapping

Examination was made of the thyroid in 94 yellow-necked field mice caught in 3 summer periods, June 1967 and 1969 and July 1966. Continued increase was found in the dimensions of this gland, and preponderance of light-staining columnar epithelium in its vesicles, and watery colloid (Fig. 8, Plate VIII). The vesicles were usually small or medium. The few very large vesicles found were of maximum length, usually exceeding  $200 \mu$ , of irregular elongated shapes and their epithelial lining consisted of relatively high cells. The epithelium protruded from the walls into their lumen, often with simultaneously narrowing of the vesicles in that place, which combined with the presence in the colloid of scattered shed epithelial cells gave the impression of these vesicles having been formed from the fusion of smaller vesicles. The walls in some of the large, and occasionally even of the medium, vesicles were divided and the colloid had penetrated between vesicles, with epithelial cells simultaneously scattered in their interior. These changes were sometimes so considerable that they made it difficult to distinguish individual vesicles (Fig. 9, Plate VIII). In the apical part of some of the columnar cells strongly PAS--positive inclusions were observed, of as much as 1 µ in diameter, probably corresponding to reabsorption of colloid. The columnar epithelial cells had a large light-coloured nucleus often exceeding 5  $\mu$  in diameter. The »reconstruction of the gland structure« described was often accompanied by a good or very good blood supply.

#### Thyroid gland in A. flavicollis

In 4 out of the 94 animals examined we observed extreme differences in histological structure of the thyroid from that described. In such cases there was distinct predominance of large vesicles, most often oval in shape, filled with usually dense basophilic coloid and lined with simple flat or cubical low epithelium, the cells of which contained dark acidophilic cytoplasm and very dark small nuclei (Fig. 10, Plate VIII). In several cases we observed in the thyroid transitional histological pictures in relation to those described above (Fig. 11, Plate VIII).

In sexually active animals differences were found in the histological structure of the thyroid in males and females, which was not the case with sexually inactive animals.

# 4. Animals from Autumn Trapping

A total of 134 yellow-necked field mice caught in 4 autumn periods. (October 1966-69) were examined. Large dimensions of this gland were still observed in some of the animals, whereas in others it was clearly smaller than in summer. The larger dimensions of the thyroid were more often found in October 1966 and 1968 than in 1967 and 1969, large and medium vesicles lined with simple cuboidal epithelium predominating in the first two periods (Fig. 12, Plate VIII). Compared with summer slightly more connective tissue was observed between the vesicles, particularly on the periphery of the gland, with an admixture of brown or yellow fat cells (Fig. 13, Plate IX). The epithelium was slightly higher in almost one third of cases, and was even columnar with large light-coloured cells, on the periphery of the thyroid (Fig. 14, Plate IX). In October 1967 and 1969 the thyroid was characterized by smaller dimensions and predominance of medium vesicles lined with higher epithelium than in previous years (Fig. 15, Plate IX). Colloid remained moderately dense and often basophilic. In addition groups of hypertrophic cells not forming vesicles were relatively often encountered in the thyroid. Where they occurred more numerously the gland was well supplied with blood, whereas this supply was slightly poorer in other places.

No sex dimorphism was observed in the structure of the thyroid in any of the four autumn periods, but moderately distinct individual variations were found, also the dependence on sexual activity to which reference has been made above.

# 5. Changes with Age in the Morphological Picture of the Thyroid

Mainly small vesicles, lined with columnar or cubical epithelium, and containing watery colloid, were found to occur in the thyroid of animals from age class I caught in summer and autumn periods only. This structure, together with the good blood supply to the gland, are evidence of its intensified endocrine activity in the youngest animals (Fig. 16, Plate IX). Changes observed in the structure of the thyroid in age classes from II to IV could only be connected with the given season, sex or sexual activity.

In the group of oldest animals (age class V) the dimensions of the thyroid decreased and simultaneously there was distinct increase in the connective tissue between the vesicles, containing relatively numerous fat cells. In all this gave a picture of involution of the gland (Fig. 17, Plate IX).

In addition to age both lactation, and in particular gestation, affect the structure of the thyroid, resulting chiefly in increase in its dimensions.

Some of the animals examined exhibited degenerative changes in certain parts of the thyroid, consisting in destruction of vesicles, probably due to necrotic changes within their epithelial lining, with simultaneous occurrence of infiltrations consisting of cells similar to lymphocytes (Fig. 18, Plate IX). These infiltrations were often very abundant and included the intervesicular connective tissue which had proliferated in these places. The frequency of occurrence of these changes, and also their extent, increased with age and were most advanced in the oldest animals, and it was only in 4-week old individuals that they were not observed at all. In addition slightly greater intensity of the changes described was observed in the thyroid of animals caught in winter than in animals of the same age, but caught at other times of the year.

# IV. DISCUSSION

We assumed that both increase in the height of vesicular cells and increase in the dimensions of their nuclei are the chief signs of increasing thyroid activity. In addition decrease in the density of colloid and hyperaemia of the organ point to intensified thyroid activity.

We also took the dimensions of vesicles into account, but found that large vesicles do not always correspond to low activity of the gland. The appearance in such vesicles of high columnar epithelium with large light-coloured cell nuclei and peripheral vacuolization of colloid suggests sudden activity of factors stimulating the functioning of the thyroid after a period of low activity. Even stronger stimuli may cause the farreaching changes in the structure of the thyroid, described in the summer periods. Similar capacity for changes in structure has been observed in the thyroid of other animals as well, e.g. Dzierżykraj-Rogalska (1952) in Sorex araneus or Vijayan & Sathyansen (1971b) in Funambulus pennanti Wroughton.

We considered characters such as proliferation of connective tissue between vesicles with simultaneous disappearance of gland tissue, and particularly distinct decrease in the dimensions of the gland, as symptoms which, in addition to the opposite characters to those described during increased activity of the gland, provide evidence of decreased endocrine activity of the thyroid gland.

Using the above criteria we established that the thyroid is subject to cyclic seasonal changes in vellow-necked field mice over 2 months old. Lowest activity was observed in winter, followed by distinct increase in endocrine activity in spring, most often unevenly distributed over the various parts of the thyroid, while maximum thyroid activity was observed in summer and its gradual reduction during the autumn. We have purposely not dealt separately in the above description with exactly defined phases of thyroid activity over the course of one yearly cycle, as our observations (despite the fact that they were not carried out continuously from month to month) indicate that these periods interconnect and that in a certain number of cases deviations from the histological pictures of this organ occur simultaneously. Some of these deviations can be explained by the occurrence or absence of sexual activity, by advanced age and to a lesser degree by the difference in sex of the animals. In other cases in which it proved impossible to ascertain the causes differences in the structure were considered to be due to individual variation.

The appearance in single cases in February 1967 of stimulations of the thyroid similar to those occurring in spring can be explained by the early onset of sexual activity in that year.

Similar results in evaluation of seasonal activity of the thyroid in free-living small mammals from the temperate zone have also been obtained by other authors: in rodents by Delost & Naudy (1956), Rigaudière & Delost (1956), Rigaudière (1968a, b, 1969), and in Sorex araneus by Dzierżykraj-Rogalska (1952), Hyvärinen (1969), in Neomys fodiens by Dzierżykraj-Rogalska (1957). The slight differences occurring in these descriptions may be due to the differences between species, and to climatic or habitat differences. In all the animals examined the thyroid was found to be least active in winter. According to Rigaudière (1969) the thyroid in voles exhibited a histologically similar cycle of seasonal changes to that observed in the thyroid of hibernating animals. She is of the opinion, after Delost (1960), that the seasonal changes observed in these animals in the peripheral endocrine glands were probably directly connected with changes in the hypothalamus-pituitary system, which exhibits significant seasonal changes and intensive summer activity.

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Dzierżykraj-Rogalska (1952) did not, however, observe histological manifestations of decrease in thyroid activity in winter in Sorex araneus, but observed considerable reduction in the dimensions of the gland, like that we found in the field mice examined. Interesting results were obtained by Eleftheriou & Zarrov (1962), who found maximum thyroid activity in Peromyscus in winter and minimum in summer.

It has been known for a long time that thyroid activity in large mammals and also in laboratory rodents differs from what we found in our studies. In the latter changes in the cycle of endocrinal function are caused by their constantly living under laboratory conditions. Mosier & Richter (1967) found that there are distinct differences in the structure and reactivity of the thyroid between wild migratory and laboratory rats. In the latter the thyroid, as shown by Piroth & Eisenbrand (1970), reacts *inter alia* by increase in its activity to the longterm effect of low temperatures.

The effect of age on thyroid structure was only apparent in the youngest and oldest individuals of Apodemus flavicollis. In all the youngest animals this gland is characterized by micro-vesicular structure, high vesicular epithelium, thin colloid and good blood supply, suggesting its intensified endocrine activity. The above results agree with the observations made by Mathur (1971) on Bubalus bubalis (L.), Bezrukov & Koževnikova (1970) in the camel and Dzierżykraj--Rogalska in Sorex araneus (1952) and Neomys fodiens (1957). In the oldest mice the thyroid was differentiated from other age classes by the occurrence of distinct involutional changes and lower (according to the period under consideration) activity to that generally observed. Reduced thyroid activity in old animals has also been found by other authors: Bezrukov & Koževnikova (1970) and Dzierżykraj-Rogalska (1952). The appearance of certain changes of an involutional character which we observed in the thyroid in some of the mice in winter can probably be explained, not only by the long-lasting low level of its activity, but also by other causes. For instance Trendelenburg (1934) states that hunger and in particular deficiency of B group vitamins cause atrophy of vesicles and reduces secretion of thyroid. Money (1955) also found that malnutrition greatly reduces thyroid activity in laboratory mice. It may therefore be assumed that the yellow-necked field mouse suffers from insufficiency in quality if not in quantity of food in winter.

The correlation we found between sexual activity and thyroid activity have only exceptionally been perceived by other authors (cf. *e.g.* Dzierżykraj-Rogalska, 1952). Bezrukov & Koževnikova

(1970) state that sexual maturation exerts a distinctly stimulating influence on the activity of this gland, basing this statement on observations of young camels. They emphasise in this connection, which agrees with our own observations, that the thyroid in males during the period of stimulation connected with sexual activity is more active than that in females. Experimental studies made by Wiśniewski & Chodynicki (1952), Wiśniewski (1960) and Chodynicki & Gutsze (1962) show that thyroid activity depends to a great extent on the gonads. When the latter are removed from adult laboratory mice thyroid activity is greatly reduced, this being most clearly seen in males.

Gestation and lactation were observed to exert a stimulating effect on thyroid activity, and this agrees with observations made by many other authors: Bezrukov & Koževnikova (1970), Tsarikovskaja (1964), Grosblat (1965), Narbutt *et al.* (1970). The occurrence in some of the females of pictures similar to endemic struma can be explained by the fact that gestation, as has been known for a long time (Górecki, 1968) may greatly intensify symptoms of hypothyroidism. It has been found that endemic struma occurs relatively frequently among animals in some regions of the Soviet Union (Bezrukov & Koževnikova, 1970).

Pathological changes of a degenerative and necrotic character, occurring in the thyroid of some of the mice examined, must be discussed separately. Similar changes were observed by Vijayan & Sathyan esan (1971a) in *Funambulus pennanti* who suggest that these changes may be caused by diet deficiencies or non-specific infections. It is very likely that they may also develop in connection with an allergy, since Käresen (1970) observed abundant infiltrations composed of lymphocytes, with the addition of other leucocytes, both in the stroma and vesicular epithelium and in the lumen of the vesicles themselves of the changed thyroid in a guinea pig in which allergic thyreoitis had been artificially caused.

To recapitulate it may be assumed, on the basis of changes in the histological structure of the thyroid, that a distinctly cyclic biological rhythm connected with the seasons of the year occurs in the thyroid activity of yellow-necked field mice. It is only altered to a certain extent by the effect of sexual activity and to a lesser degree by the sex and age of the animals. The individual deviations described in the text in the histological structure of the thyroid in different field mice, and also between whole groups of these animals caught at the same seasons of the year but in different years, can be explained by the great susceptibility of the thyroid to any strong stimulus, whether exogenic or endogenic.

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# BUDOWA MIKROSKOPOWA TARCZYCY MYSZY WIELKOOKIEJ LEŚNEJ APODEMUS FLAVICOLLIS (MELCHIOR, 1834) W CYKLU ROCZNYM

#### Streszczenie

Zbadano tarczycę 265 myszy wielkookich leśnych, pochodzących z wyłowów prowadzonych w różnych porach roku, w Białowieskim Parku Narodowym w latach 1966-1969. W podziale na grupy uwzględniono płeć, wiek i aktywność płciową, a u samic także okres ciąży i laktacji. W wieku około 4 tygodni stwierdzono u obu płci jednakową budowę histologiczną tarczycy odpowiadającą wzmożonej czynności dokrewnej (Fig. 16, Tablica IX) oraz brak zmian sezonowych. W wieku od 2 miesięcy do 1 roku wystąpiła wyraźna zmienność sezonowa budowy tarczycy. Najniższą aktywność obserwowano w zimie (Fig. 3, 4, Tablica VII) na wiosnę występował wyraźny wzrost czynności dokrewnej (Fig. 5, 6, Tablica VII) maksimum aktywności tarczycy obserwowano w lecie (Fig. 8, 9, Tablica VIII), a stopniowe jej obniżenie w okresie jesiennym (Fig. 12, Tablica VIII). W materiale pochodzacym od zwierząt powyżej 1 roku pojawiły się w gruczole tarczowym zmiany inwolucyjne. (Fig. 17, Tablica IX). Podobne zmiany występują sporadycznie również u zwierząt w wieku poniżej 1 roku, ale tylko w okresie zimowym. Zaobserwowano wpływ aktywności płciowej na zmiany w budowie tarczycy. Ciąża jak i laktacja prawie zawsze działały pobudzająco na czynność gruczołu tarczowego. Obserwowano również różnice osobnicze w obrazach morfologicznych tarczycy zwierząt należących do jednakowych grup.

# EXPLANATION TO PLATES VII-IX

Fig. 1. Part of the thyroid from a sexually inactive male caught in December. Proliferation of brown fat, within which there are single thyroid vesicles, can be seen on the periphery of the gland. Magn. approx. 180×.

Fig. 2. Part of the thyroid of a sexually inactive male caught in Decemebr. Low or flat cubical epithelium can be seen in small vesicles. Magn. approx.  $370 \times$ .

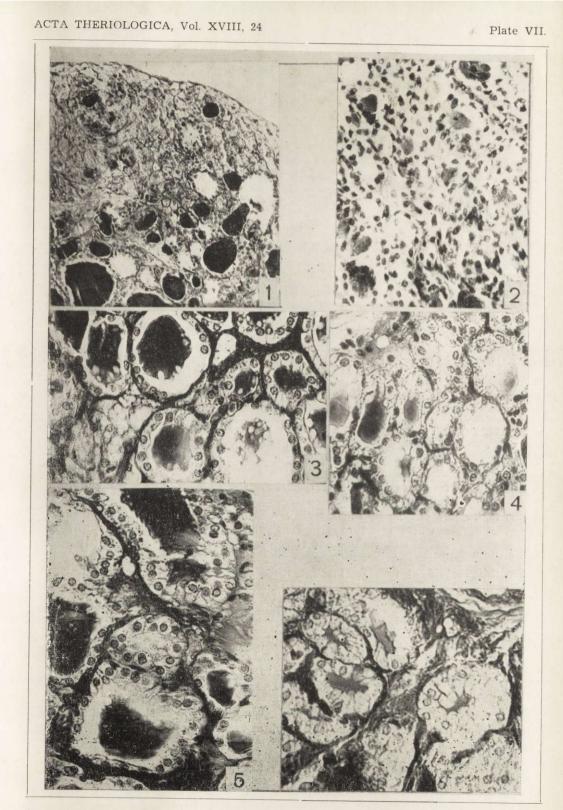
Fig. 3. Thyroid of a sexually active male caught in February. Ephithelial cells of varying height and marginal vacuolization of colloid in vesicles can be seen. Magn. approx. 370×.

Fig. 4. Thyroid of a sexually active female caught in February. Cells of vesicular epithelium are of uniform height. Magn. approx. 370×.

Fig. 5. Thyroid of a sexually active male caught in April 1966. Increase in the height of vesicular epithelium cells applies to large vesicles also. Magn. approx.  $370 \times$ .

Fig. 6. Thyroid of sexually active male caught in April 1966. High columnar vesicular epithelium can be seen in the whole gland, also watery colloid and intensive blood supply. Magn. approx. 370×.

Preparations from thyroid glands were fixed in Bouin's fluid and stained by the Azan method.



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#### Plate VIII.

Fig. 7. Thyroid of sexually active male caught in April 1967. Variations can be seen in the height of the vesicular epithelium. Magn. approx.  $370 \times$ .

Fig. 8. Thyroid of sexually active male caught in July. Part of the thyroid exhibits characters of morphological structure typical of the summer stimulation period. Magn. approx.  $370 \times$ .

Fig. 9. Thyroid of sexually active male caught in July. High epithelium and watery colloid can be seen in vesicles. Magn. approx.  $370 \times$ .

Fig. 10. Thyroid of sexually inactive male, caught in Jule 1969. Large and very large vesicles lined with flat epithelium and filled with dense colloid can be seen. Magn. approx.  $370 \times$ .

Fig. 11. Part of the thyroid of sexually active male caught in July. In large and very large vesicles flat epithelium and basophilic colloid can be seen. Magn.  $approx. 370 \times .$ 

Fig. 12. Thyroid of sexually active male caught in October 1966. Cubical epithelium and watery colloid can be seen in the vesicles. Magn. approx.  $370 \times$ .

# Plate IX.

Fig. 13. Part of the thyroid of a sexually inactive male caught in October 1968, its strongly development fatty tissue between vesicles. Magn. approx  $370 \times$ .

Fig. 14. Thyroid of sexually active male caught in October 1966. Peripheral distribution of vesicles with higher epithelium can be seen. Magn. approx.  $370\times$ .

Fig. 15. Thyroid of sexually active male caught in October 1969. Higher epithelium occurs in the whole gland as compared with thyroids of animals caught in October 1966 and 1968. Magn. approx.  $370 \times$ .

Fig. 16. Thyroid of sexually inactive male caught in June, belonging to age class I. The small dimensions of vesicles with higher epithelium are characteristic. Magn. approx.  $370 \times$ .

Fig. 17. Thyroid of sexually active lactating female from age class V, distinguished by clearly visible manifestations of involution in the form of strong proliferation of connective tissue in the place of disappearing glandular tissue. Magn.  $approx. 80 \times .$ 

Fig. 18. Thyroid of sexually active male caught in April 1966. Abundant infiltrations can be seen, composed of lymphocyte-like cells occupying both intervesicular connective tissue and lumen of vesicles. Magn. approx. 370×.

Plate VIII.



auctores phot.

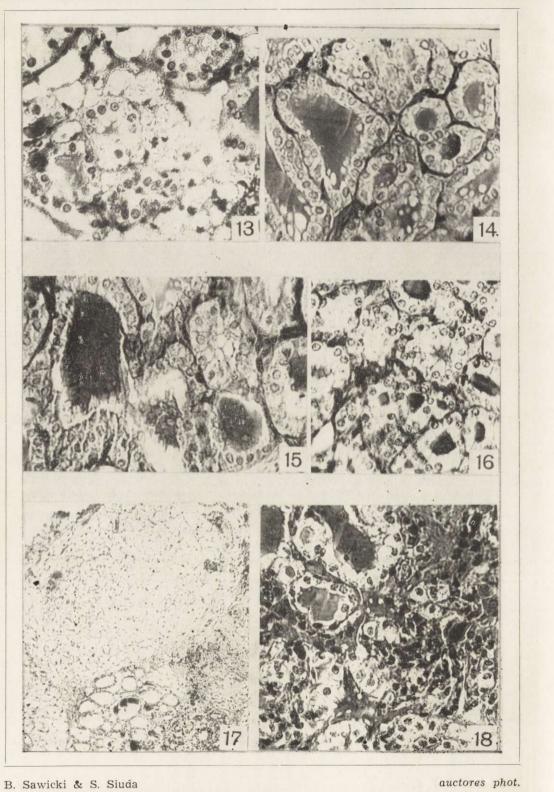


Plate IX.