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**Morphology and Histochemistry of Thyroid Gland C Cells
of Young and Adult Guinea Pigs**

[With 2 Tables & Plates V—VII]

Examination was made of C cells in the thyroid gland of 32 male guinea pigs, 14 of which were 2—3 months old and 18 were 1—2 years old. It was found that the following staining methods can be used as markers of thyroid C cells in these animals: Grimelius's silver impregnation, HCl-toluidine blue and HCl-lead hematoxyline, besides reactions to nonspecific esterases and cholinesterases. Thyroid C cells in the guinea pig are distributed irregularly and occur both singly and in clusters of varying size. They are usually arranged in the epifollicular position, less often in the intraepithelial and most rarely in the interstitial position, and are characterized by distinct polymorphism. An average of 8.1% of C cells were found in the thyroid gland of young guinea pigs and 13.2% in adult animals. In young individuals they occur in smaller groups and exhibit lesser fluctuations, both in respect of the amount of cytoplasmatic granules and in activity of acid phosphatase, than is the case with adult animals.

I. INTRODUCTION

In recent years after thyroid C cells (parafollicular) in mammals had been proved to produce the hormone calcitonin, the number of publications concerned with physiological or pathological changes forming in the structure of the cells under different experimental conditions has increased. Studies are usually made on white rats and dogs, and consequently the morphology and histochemistry of C cells has been studied in the greatest detail in these animals, whereas in other species thyroid C cells have received less attention. I have not encountered a detailed description of these cells in so common a laboratory animal as the guinea pig in the literature to which I had access. An attempt has been made in the present paper to give a detailed description of the morphology and histochemistry of thyroid C cells in young and adult guinea pigs, as it is assumed that the different metabolism of calcium ions in these age groups may be reflected in the morphology of C cells.

II. MATERIAL AND METHODS

The material used for the studies consisted of 32 male guinea pigs, 14 of which were 2—3 months and 18 were 1—2 years old. All the animals were kept under uniform laboratory conditions, and were killed under anaesthesia by exsanguination. Blood was taken from some of the animals before they were killed, in order to determine serum calcium concentration. The thyroid gland was fixed in Bouin's, GPA (Solcia & Sampietro, 1968) and Baker's fluids. The following histological and histochemical methods were used in the studies: 1) hematoxylin-eosine; 2) Azan; 3) Grimelius silver impregnation (Sawicki & Bajko, 1974); 4) HCl — lead hematoxyline (Solcia *et al.*, 1969); 5) HCl — toluidine blue (Sawicki, 1971); 6) reaction to succinate dehydrogenase (*SD*) after Nachlas *et al.* — incubation time 30 minutes (Pearse, 1960); 7) reaction to α -glycerophosphate dehydrogenase (α -*GPD*) — incubation time 20 minutes (Wattenberg & Leong, 1960); 8) reaction to nonspecific cholinesterase (*PCh*) — incubation time 8 to 12 hours, using acetylo- or butyrylthiocholine iodide as substrates (Karnovsky & Roots, 1964); 9) reaction to nonspecific esterase (*NE*) by the method of combining with diazo-salts: Salt no. 2, 9 and 18 — produced by EG England, substrates — AS-naphthol acetate or α -naphthyl acetate, incubation time 5 minutes (Pearse, 1960); 10) reaction to acid phosphatase (*APh*) after Burstone — method of combining with diazo-salt No.18, incubation time 1 hour (Pearse, 1960).

Enzymatic reactions were made on 10—12 μ cryostatic sections taken from unfixed thyroid glands (when dehydrogenases were used) or thyroid glands fixed in Baker's fluid at a temperature of +30°C (when esterases were used). Paraffin sections 6 μ thick were used in all the other stainings.

Incubation of part of the sections examined without substrates was introduced as a check on all enzymatic reactions. When esterases were studied ezerine was used additionally in control reaction and control reaction to lipases was carried out by Tweens' method (Pearse, 1960).

Total and ionized calcium concentrations in serum were measured by means of a flame photometer. The resulting measurements were analysed statistically, using Student's test (Ruszczyk, 1955).

The approximate percentage of thyroid C cells was calculated for 23 animals. Calculations were made on 20 sections from each lobe of the thyroid gland sectioned serially, choosing the sections with greatest cross-section area for the purpose, and counting C cells in every second section in preparations treated with Grimelius' silver impregnation. Arithmetical averages and medians of the percentage of thyroid C cells in the two study groups were calculated from the results obtained.

III. RESULTS

1. Morphology and Histochemistry

It is not possible to distinguish with absolute certainty thyroid C cells from follicle cells (thyrocytes) in young guinea pigs after staining thyroid sections by the Azan or hematoxyline-eosine methods. In histological preparations of adults thyroid glands stained with the same methods it is, however, possible to see relatively large clusters of cells morphologically slightly different from follicle cells. Preparations silver-impreg-

nated by Grimelius' method, then desilvered and stained by the Azan method, show that these are thyroid C cells (Fig. 1, 2). It is very difficult accurately to identify C cells lying singly in adult animals also (Fig. 3). The clusters of C cells referred to are similar in appearance to syncytium, on account of the rarely perceptible boundaries between the cells. This is as a rule the case when cell nuclei occur densely near each other. The boundaries between C cells and follicle cells are always clearly visible.

The most characteristic feature distinguishing C cells from follicle cells is the structure of their nuclei (Fig. 3). These nuclei are most often almost round or oval and vary considerably in dimensions. In addition to very much larger nuclei, it is also possible to find nuclei similar in size to those observed in thyrocytes. A certain small number of C cells have even smaller nuclei. Small nuclei sometimes occur in the central parts of some larger clusters of these cells (Fig. 5), but small nuclei are very rarely encountered in C cells lying singly or in small clusters. The next significant feature of the nuclei of C cells is the occurrence in them of large basophilous granules — usually 2 to 3 (Fig. 2, 3). They are similar to the nucleoli of thyrocytes in respect of their position on the internal area of the nuclei, but have less regular shapes. In addition basophilous granules occur in the nuclei of C cells smaller than those described and are most often situated near the nuclear membrane; they are similar to the chromatine granules of follicle cells but are more numerous and larger. Differences in readiness of staining and cytoplasm structure of these two types of thyroid cells are slight and most often not even noticed.

In young guinea pigs the morphological differences described above between C-cells and follicle cells (visible in preparations stained with the above methods) are not too distinct, although the general plan of C cell structure is the same as in adult animals. This is caused chiefly by the more deeply staining nuclei of follicle cells, possessing larger chromatine granules than those observed in the analogical cells in adult animals.

After selectively distinguishing thyroid C cells in the study animals by means of impregnation with salts of silver, metachromatic staining with toluidine blue or staining with lead hematoxyline, it was found that they are more numerous in adult animals (Table 1).

Some of the C cells in the guinea pig occur in the form of larger clusters, such groups of cells being far more frequent and larger in adult animals (Fig. 8). In young animals we more often encounter single C cells (Fig. 9).

The largest number of C cells are revealed by Grimelius' silver impregnation, since this permits of observing the structure of the cell

nuclei (Fig. 1, 7), and gives a dark colour to the cytoplasm even in cells possessing little cytoplasmic granular matter (Fig. 5). With the other two methods of selective staining of thyroid *C* cells the cell nuclei remain unstained, and it is therefore practically impossible to recognize *C* cells with scanty granular matter, especially those lying singly (Fig. 11). The coarse granular structure of *C* cell cytoplasm is visible with all the above-mentioned methods of staining. When there are few granules in the cytoplasm they can be seen more clearly with the silver impregnation method (Fig. 5), which also renders the cell shapes clearer. In general thyroid *C* cells, whether in young or adult guinea pigs, are characterized by polymorphism which is most clearly seen when they occur singly. Polymorphism is due both to the occurrence in *C* cells of short cytoplasmic extensions (Fig. 4, 10) and to the different dimensions of the various cells, and depends on change in shapes resulting from the variety exhibited by the position of these cells in the gland. Another feature characteristic of guinea pigs is the varying amount of cyto-

Table 1

Contents in percentage of *C* cells in the thyroid gland.

Age group	No. of animals	Avg. \pm S.D.	Median	Observed range, %
Young (2—3 months old)	11	8.1 \pm 2.5	7.5	5—14
Adult (1—2 years old)	12	13.2 \pm 1.8	13.0	11—17

plasmatic granules in different *C* cells. Cells with an extremely large amount of granular matter occur side by side with cells with more or less distinctly smaller amounts of these granules, up to complete absence of such granules in the cytoplasm. The range of variations in cytoplasmic granule content is smaller in young than in adult animals.

Observation of successive series of sections shows that the most frequently found position of *C* cells in the thyroid of the guinea pig is the epifollicular (Fig. 12), the intraepithelial position occurring far less often. Part of the cells arranged in this way give the impression that they may be in direct contact with follicular colloid, particularly when the follicle lining is thin (low) (Fig. 1, 6). The interstitial position of thyroid *C* cells is most rarely observed (Fig. 7).

Of the enzymatic reactions carried out, the least selective proved to be reaction to *SD*, since we only occasionally observed groups of *C* cells which were distinguished by slightly stronger enzymatic activity (Fig. 13) than was observed in thyrocytes. It was found from the intensity of reaction that *SD* activity is small in both *C* cells and follicular cells in the thyroid of both young and adult guinea pigs.

In reaction for α -GPD a strong or very strong positive reaction was found in C cell cytoplasm, but intensivity of this enzymatic reaction in thyrocytes increases with the height of the cells. Low or very low enzymatic activity is observed in flat follicular epithelium whereas in cubical epithelium it varies from moderate to strong. Under these conditions C cells are clearly distinguishable only in the vicinity of follicles lined with flat epithelium (Fig. 14); parts of the thyroid of this type, however, are only sporadically encountered in adult guinea pigs. Usually differences in enzymatic activity in these two types of cell are slight and permit of identifying with a high degree of probability only large clusters of C cells. It is even more difficult to identify C cells in young guinea pigs from reaction to α -GPD, on account of the slight differences in intensity of reaction in the two types of cell (Fig. 15).

Reaction to NE with AS-naphtol acetate as substrate is weakly positive both in thyrocytes and C cells, whereas reaction with α -naphthyl acetate is weakly positive in thyrocytes and strongly or very strongly positive in the majority of C cells in both young (Fig. 17) and adult animals. Only a few C cells exhibit moderately positive reaction, and consequently it is possible to distinguish C cells even when occurring singly. It must be emphasised here that distinct increase in NE activity, rendering identification of C cells situated nearby difficult, is observed in raised thyrocytes, but parts of this type rarely occur in the thyroid gland.

In reaction to PCh, both when using acetylthiocholin iodide or butyrylthiocholine iodide as substrates, there is a strongly positive reaction in the majority of thyroid C cells in young and adult guinea pigs (Fig. 18). Some of cells or groups of them are even distinguished by very strongly positive enzymatic reaction, only a few C cells being characterized by weak or moderate reaction to PCh. The weak positive reaction to PCh simultaneously found in thyrocytes is subject to practically no fluctuations; it would seem that its intensivity is independent of the thickness of the epithelium lining the follicles. When using the same sections for reaction to NE and then to PCh the products of these two reactions are observed to become deposited in the same cells (C cells).

Reaction to APh in adult guinea pigs is most often weakly positive in the majority of thyrocytes. The remaining thyrocytes usually have a strongly positive reaction, situated chiefly in the apical part of cytoplasm. C cells can be identified with certainty in this reaction only within certain of the large clusters (Fig. 19) lying in the vicinity of thyrocytes with low enzymatic activity. In general APh activity differs greatly in different C cells, as it does in thyrocytes. In the latter reaction to APh markedly intensifies with increase in cell dimensions.

In young guinea pigs a slightly stronger reaction occurs in the reaction to *Aph* in follicular cells than that observed in adult animals, and far smaller fluctuations in the intensity of this reaction in *C* cells. Thus *C* cells cannot be identified with certainty in the thyroid of young guinea pigs by using this reaction.

All the methods used to reveal *C* cells showed that these cells are unevenly distributed in the thyroid gland of the guinea pig — in places they are very numerous, or they may not occur at all in places. It is particularly the external zone of the gland, varying greatly in width, which is completely devoid of them.

2. Serum Calcium Concentration

Results of measurements of serum ionized calcium concentration and total calcium concentration in the animals studied are given in Table 2. The analysis made shows that no statistically significant differences

Table 2

Serum calcium concentration — total and ionized in young and adult guinea pigs.

Age group	No. of animals	Average \pm S.D., mg‰	
		Ionized calcium	Total calcium
Young (2—3 months old)	9	5.20 \pm 0.57	10.04 \pm 1.05
Adult (1—2 years old)	10	6.42 \pm 1.26	10.89 \pm 1.77

occurred in total calcium concentration between the groups examined, only deviations in ionized calcium concentration proving to be statistically significant ($p < 0.05$).

IV. DISCUSSION

The majority of contemporary authors distinguish 3 main types of epithelial cells in the thyroid gland. The follicular cells which, as is well known, produce thyroxine and its derivatives, were the first cells to be identified, and were followed by the identification of thyroid *C* cells as »perenchymal cells« (Baber, 1976) and were in turn described in a large number of species under a wide variety of names (Sawicki, 1972a). The contradictions occasionally occurring in descriptions of these cells form evidence that it was not always true *C* cells which were in question (Lietz & Zippel, 1969). This was due to the difficulties encountered when identifying them by means of the basic methods of histological staining (Sawicki, 1972b). It was found that the so-called »light cells« of the thyroid gland in both histochemical and immunofluorescent studies do not always correspond to *C* cells and *vice versa*, the

latter are not always light cells (Kracht *et al.*, 1969). The present interest in C cells is connected with the hypothesis that they are capable of producing calcitonin (Foster *et al.*, 1964). This assumption has been confirmed in immunofluorescent studies (Busolatti & Pearse, 1967; Kracht *et al.*, 1967), and recently also in cytoimmunochemical studies (De Grandi *et al.*, 1971; Kalina & Pearse, 1971), finding calcitonin in the cytoplasmatic granular matter characteristic of C cells. Askanazy's cells (Askanazy, 1898), which occur in the human thyroid in pathological states of this gland and in senescence, were the last to be identified. Although it is assumed that these cells may also exist in animals (Alešín, 1973) this has not as yet been fully documented. The frequently used name Hürthle's cells, instead of Askanazy's cells, is incorrect as Hürthle (1894) described typical C cells in his paper on the thyroid gland in the dog. The function of Askanazy's cells and their origin have formed the subject of numerous studies and contradictory hypotheses (Beskid & Kobuszevska-Faryna, 1972; Katelbant-Balasse & Nève, 1974; Mikhailov, 1972; Raikhlin & Mikhailov, 1974; Rudnitskaya, 1970).

There are only a few reports comparing the number of C cells with the number of follicular cells in the thyroid gland in mammals. The small number of C cells which they present as found in the thyroid gland of the mouse (Marks, 1969), rat (Thompson *et al.* 1962) and rabbit (Lees, 1970) must give rise to doubt as it is only differences in their morphology which have been taken as a basis for identifying the two types of cell.

Only Kameda (1968) counted C cells after their relatively specific silver impregnation by Davenport's method. She succeeded in establishing that there are approximately 30 C cells to 100 follicular cells in the rat, 15 in the mouse, guinea pig, rabbit and from 30 to 90 in the dog. These results, in the case of the guinea pig, correspond, after conversion into percentages, to the number of C cells which I calculated to occur in the thyroid gland of adult guinea pigs.

On the other hand Youngs *et al.* finding (1968) that the thyroid contains only about 1% of C cells in the pig may be due both to species differences between these animals and to the low degree of specificity of Cajal's silver impregnation method used by these authors to identify C cells (Sawicki & Bajko, 1974).

In studies by other authors we find only attempts at a general comparative estimate of the number of thyroid C cells in the mammals examined. It was established in this way that there are more C cells in rodents than in the *Primates* (Lietz & Zippel, 1969; Solcia *et al.*, 1970).

In rats of advanced age there may even be hyperplasia of C cells, changing with time into the so-called gamma-tumours (Lietz, 1971). The lower percentage content of thyroid C cells which I found in young guinea pigs than in adult animals agrees with the observations of Solcia & Sampietro (1968) who found that in newborn guinea pigs and rabbits C cells are less numerous than in adult animals. There are also supposed to be fewer C cells in young rats (Lietz, 1971). The different course of metabolism in the bone tissue of young animals may explain the latter's smaller calcitonin requirements, as the growing bone, by means of intensive calcium intake, prevents the states of transitory food hypercalcemia encountered in adult animals (Haas *et al.*, 1972).

The different results obtained from comparison of the number of C cells in young and adult individuals in the case of dogs (Kameda, 1971) and humans (Kalina *et al.*, 1970) may be due to the fact that, for instance in newborn humans these cells are very distinctly clustered near the »external parathyroids« (Solcia *et al.*, 1970), which may give the impression of their numbers being greater when observing such parts of the thyroid gland in children. Taking into account the above fact, and also the usually very uneven distribution of C cells in the human thyroid (Kalina *et al.*, 1970; Solcia *et al.*, 1970) examined here in small sections on account of its large dimensions, it may be assumed that such an approximate comparative estimate of the number of C cells between sections of the thyroid gland in adult humans and children is very unreliable.

Despite the existence of individual variation in the total number and distribution of C cells (Lietz, 1971; Sawicki, 1972a) their number, topography and morphology often differ sufficiently in various mammals to become characteristic of them. Thyroid C cells in the guinea pig also (after being specifically stained) differ distinctly from the C cells of the rat or mouse (Sawicki, 1972b), although these are animals belonging to the same order (*Rodentia*). Although C cells most often occur in the epifollicular position in the guinea pig, as they do in the other mammals so far examined in this respect (Lietz, 1971), yet it comparatively often happens that a different localization of C cells is observed in relation to the thyroid follicles. All the foregoing, together with the C cell polymorphism previously described, gives a microscope picture characteristic of the guinea pig.

It must be stressed that considerable differences of opinion have hitherto existed among researchers as to the types of possible localization of thyroid C cells in mammals, and furthermore it is even more difficult to bring such view into line owing to the variety of the nomenclature often used. The majority of authors consider, for instance, that only the epifol-

licular position of thyroid C cells occurs in the rat (Azzali, 1968; Ekholm & Ericson, 1968; Kristić, 1969), without taking into consideration their intraepithelial position, and definitely deny the possibility of direct contact existing between C cells and follicular colloid. Detailed observations made on semi-thin sections, and the picture observed under an electronic microscope, show that contact between C cells and colloid is only apparent (Lietz *et al.*, 1969; Velický, 1970), nevertheless cases have been observed under an electronic microscope of direct contact of C cells with follicular colloid (Dörrenhaus *et al.*, 1971; Stoeckel & Porte, 1967; Tashire, 1964).

The interstitial position of C cells often observed in the thyroid gland of many mammals with the aid of a light microscope (Carvalho & Pearse, 1967; Gabe & Martoja, 1969; Salzer, 1971) has also caused considerable controversy, for instance some researchers have given their opinion, based on pictures seen under an electronic microscope, that there is no such localization of these cells (Biddulph & Maibence, 1972; Lietz, 1971), while others confirm observations made with a light microscope (Fetter & Capen, 1972; Redigier, 1973).

The data presented show that C cells may occupy as many as three positions in relation to gland follicles in the thyroid of certain mammals. My observations show that the guinea pig should be included among these mammals, and it would appear unjustifiable to hold that C cells occur solely in the epifollicular position in this animal (Lietz, 1971).

There is now a general conviction that with the common staining methods C cells are visible in the thyroid as lighter and larger than follicular cells. My own observations have led me to the conclusion that it is primarily intensively secreting C cells occurring in the vicinity of follicles lined with epithelium corresponding morphologically to weak thyroid function (flat or low cubical epithelium) which are distinguishable in this way. The very different dimensions of various C cells found in the guinea pig, and also their different cytoplasmic granular matter content, were probably due to the differing functional state of these cells. Very small C cells, with small nuclei and a small amount of cytoplasm poor in granular matter, were probably in a state of functional rest. Large C cells with cytoplasm completely filled with granular matter were probably in the phase of storing the hormone. Large or medium cells containing large light nuclei and little cytoplasmic granular matter probably intensively liberated calcitonin into the blood stream. C cells observed in the thyroid of guinea pigs were most often in states intermediate between the above three phases of activity then, in order of frequency of occurrence, came cells in the storing phase. The assump-

tion as to the differing functional state of different C cells is confirmed by the enzymatic studies made in which different intensity of the enzymatic reactions examined were found in different cells.

In analysing the usefulness of the methods used for C cell identification it must be emphasised that decidedly the best results were obtained by Grimelius' silver impregnation method (Sawicki & Bajko, 1974). Good results were also obtained by staining with toluidine blue (Sawicki, 1971), and slightly fainter staining with lead hematoxylin (Solcia *et al.*, 1969). The reaction with iron colloid (after hydrolysis in HCl), effectively revealing thyroid C cells in some animals (Lietz & Zippal, 1969; Roszkiewicz, 1973), do not afford the same possibilities in the case of the guinea pig. A positive reaction was obtained in both C and follicular cells. The results I obtained with MacManus' method (PAS) — staining polysaccharides — were also far from encouraging.

In the enzymatic reactions made in the thyroid of the guinea pig reactions to PCh and NE as C cell markers are worth recommendation, as in the case of certain mammals examined up to the present (Carvalhoira & Pearse, 1967; Miętkiewski *et al.*, 1973). It must be emphasised that with the guinea pig there is no necessity to use the »E-600« preparation as an inhibitor of the esterases contained in follicular cells recommended for studies made on other mammals (Carvalhoira & Pearse, 1967). The very strongly positive reaction to α -GPD, found earliest in C cells in the dog (Foster *et al.*, 1964), was later accepted by Pearse (1966) as a C cell marker in other mammals also, but in studies made on such rodents as the rat (Birov, 1971; Stachura, 1971), rabbit (Miętkiewski *et al.*, 1973) and guinea pig (Birov, 1971) this reaction was found to be completely useless as a C cell marker. In my experiment I observed that if flat functionally inactive epithelium occurred in the thyroid of a guinea pig in the gland follicles near C cells then these cells are clearly distinguishable owing to the strongly positive reaction to α -GPD in relation to the enzymatically weakly active follicular cells. I consider that the negative results of search for C cells by means of this reaction in certain rodents is due to the considerable activity of follicular cells under the conditions of the studies made. The thyroid follicles under physiological conditions are usually lined with thick epithelium in these animals. These assumptions are confirmed by Birov's studies (1971), in which considerable increase in α -GPD activity was found in the follicular cells of the thyroid in rats and guinea pigs following application of TSH, and marked decrease in the activity of this enzyme, with simultaneous reduction in the height of the follicular epithelium, following thyroxin. My observations also

show that *NE* activity in follicular cells clearly depended on functional stimulation of these cells. The observed increase in intensity of reaction to *NE*, however, occurring parallel to increase in height of the follicular cells, was not so abrupt as in the case of reaction to *α-GPD*, and consequently it made identification of *C* cells less difficult. In general, however, reaction to *NE* is also the best marker of *C* cells when the activity of the thyroid gland is low. This observation also applies to the other enzymes studied. With very low follicular epithelium it is possible to distinguish enzymatically more active *C* cells both in reaction to *SD* and *Aph*. Pictures of this type are, however, very rarely observed, so that neither of these enzymatic reactions can be treated as *C* cell markers. The considerable *Aph* activity which I found in some of the large clusters of *C* cells in adult animals requires further discussion. According to Lietz's observations (1971) this enzyme probably participates in the decomposition of excessive stores of calcitonin. This author observed strong *Aph* activity only in some of the *C* cells in mice, while in other mammals he found lesser activity of this enzyme in *C* cells than in follicular cells. The results of the studies presented here show that considerable *Aph* activity in some *C* cells can be observed in the guinea pig. As this related to adult animals and to those in which clusters of *C* cells completely filled with granular matter had been found to occur, I assume that it is in fact a question here of liquidation of any excess of the hormone produced. This is evidence of the irregular functioning of *C* cells in adult animals, which may be due to the greater fluctuations in serum calcium concentration revealed in these animals (Table 2). In addition the significantly lower ionized calcium concentration in the serum of young animals than in adults shows that the former are less susceptible to factors causing hypercalcemia. Consequently *C* cells in young animals are not so functionally burdened as they are in adults and consequently function more evenly.

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MORFOLOGIA I HISTOCHEMIA KOMÓREK C-KALCYTONINOTWÓRCZYCH TARCZYCY MŁODYCH I DOROSŁYCH ŚWINEK MORSKICH

Streszczenie

Zbadano komórki C w tarczycy 32 samców świnek morskich, w tym 14 w wieku 2—3 miesięcy i 18 w wieku 1—2 lat. Najlepsze wyniki w rozpoznawaniu komórek C w tarczycy świnki morskiej uzyskano stosując srebrową met. Grimeliusa; dobre wyniki otrzymano również w metodach barwienia HCl-błękit toluidyny i HCl-he-

matoksyлина ołowiowa. Z odczynów enzymatycznych najskuteczniejszymi wyznacznikami komórek C u świnki morskiej były reakcje na niespecyficzne esterazy i cholinesterazy. Natomiast odczyny na dehydrogenazę α -glicerofosforanową, dehydrogenazę bursztynianową i fosfatazę kwaśną wyznaczały mniej wyraźnie komórki C, i to tylko w warunkach małej aktywności gruczołu tarczowego.

Z przeprowadzonych badań wynika, że komórki C w tarczycy świnki morskiej są rozmieszczone nieregularnie i występują zarówno pojedynczo, jak i w różnej wielkości ugrupowaniach. Względem pęcherzyków tarczycy zajmują one najczęściej pozycję epifollikularną, rzadziej pozycję intraepitelialną i najrzadziej pozycję interstycjalną (interfollikularną). Poza tym komórki te charakteryzują się wyraźnym polimorfizmem w postaci bardzo zmiennych kształtów i wymiarów komórkowych.

U młodych świnek morskich w środkowych skrawkach tarczycy występują komórki C w ilości 5—14% — średnio 8,1%, a u dorosłych w ilości 11—17% — średnio 13,25%. U zwierząt młodych jest nie tylko mniej komórek C niż u dorosłych, lecz również występują one w mniejszych ugrupowaniach i wykazują mniejsze wahania w zawartości cytoplazmatycznych ziarnistości oraz w aktywności fosfatazy kwaśnej. Przypuszczalnie wynika to z odmiennego metabolizmu wapnia u zwierząt młodych, jeszcze rosnących, w porównaniu ze zwierzętami dorosłymi; przemawia za tym istnienie statystycznie istotnej różnicy w poziomach wapnia zjonizowanego w surowicy między tymi grupami wiekowymi.

EXPLANATION OF PLATES

Plate V.

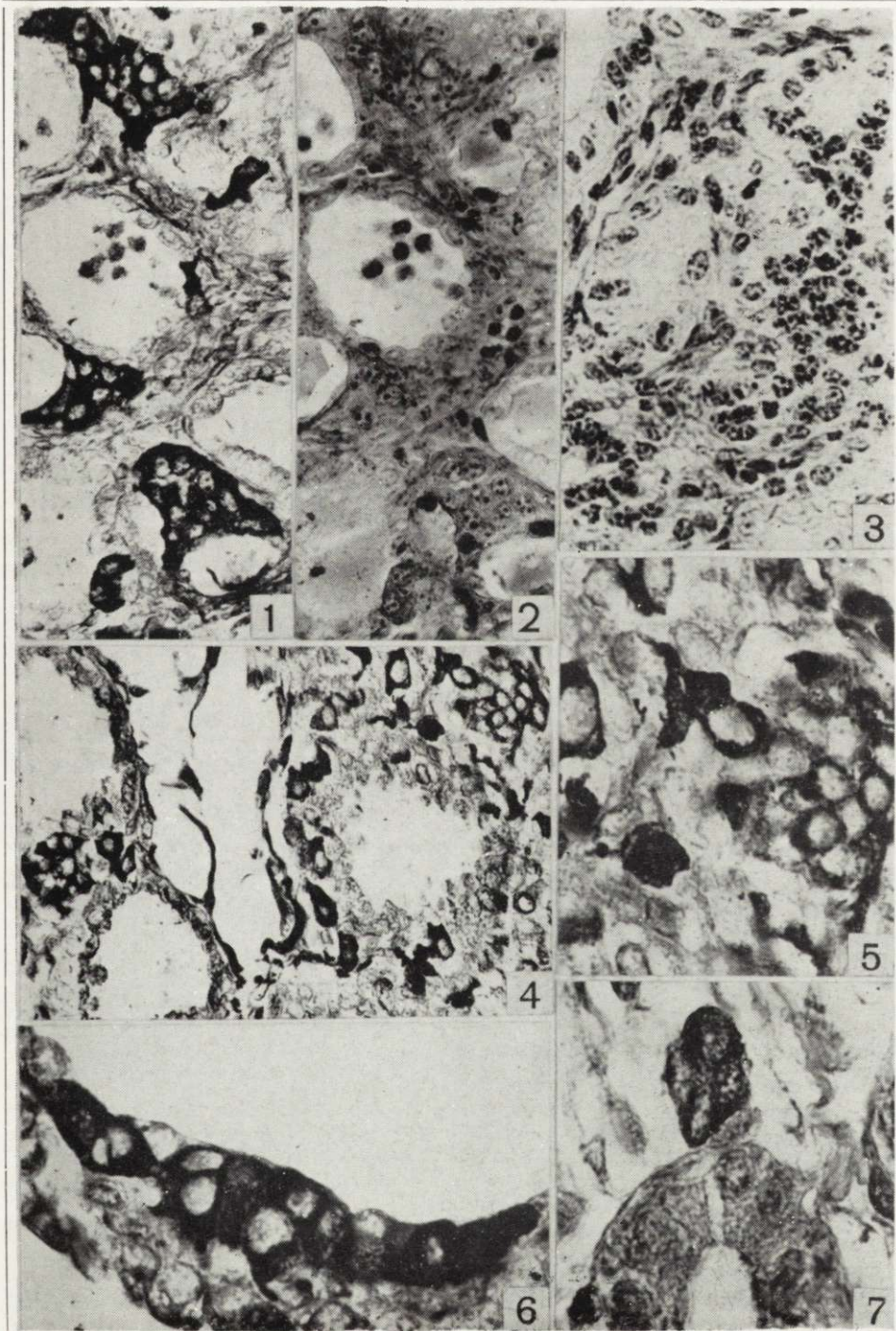
- Fig. 1 Section of the thyroid of an adult guinea pig. C cells can be seen in the form of large clusters after silver impregnation by Grimelius' method. Magn. $\times 400$.
- Fig. 2. The same section of the thyroid as in fig. 1, after desilvering and staining by the Azan method.
- Fig. 3. Section of the thyroid of an adult guinea pig. Large clusters of C cells distinguishable from follicular cells by the different structure of their nuclei. Staining by the Azan method. Magn. $\times 400$.
- Fig. 4. Section of the thyroid of an adult guinea pig, showing polymorphism of C cells. Staining by Grimelius' method. Magn. $\times 400$.
- Fig. 5. Fragment of fig. 4. Group of C cells in which cells differ in respect of the cytoplasmic granules they contain. Magn. $\times 1000$.
- Fig. 6. Fragment of a thyroid follicle in an adult guinea pig showing intraepithelial position of C cells. Staining by Grimelius' method. Magn. $\times 1000$.
- Fig. 7. Section of the thyroid of an adult guinea pig with interstitial localization of C cells. Staining by Grimelius' method. Magn. $\times 1000$.

Plate VI.

- Fig. 8. Section of the thyroid of an adult guinea pig. Exceptionally large clusters of C cells can be seen. Some of the C cells have very small metachromatically staining granules in the cytoplasm. Staining with HCl toluidine blue. Magn. $\times 250$.
- Fig. 9. Section of the thyroid of a young guinea pig. C cells occur either singly or in small clusters. Staining by Grimelius' method. Magn. $\times 400$.
- Fig. 10. Section of the thyroid of a young guinea pig, showing cytoplasmic extensions in C cells. Staining by Grimelius' method. Magn. $\times 1000$.
- Fig. 11. Section of the thyroid of a young guinea pig. Staining with HCl-toluidine blue. Magn. $\times 500$.
- Fig. 12. Section of the thyroid of a young guinea pig. The majority of C cells can be clearly seen in the epifollicular position. Staining with lead hemotoxyline. Magn. $\times 500$.
- Fig. 13. Section of the thyroid of an adult guinea pig. Reaction to succinate dehydrogenase (SD). Magn. $\times 750$.

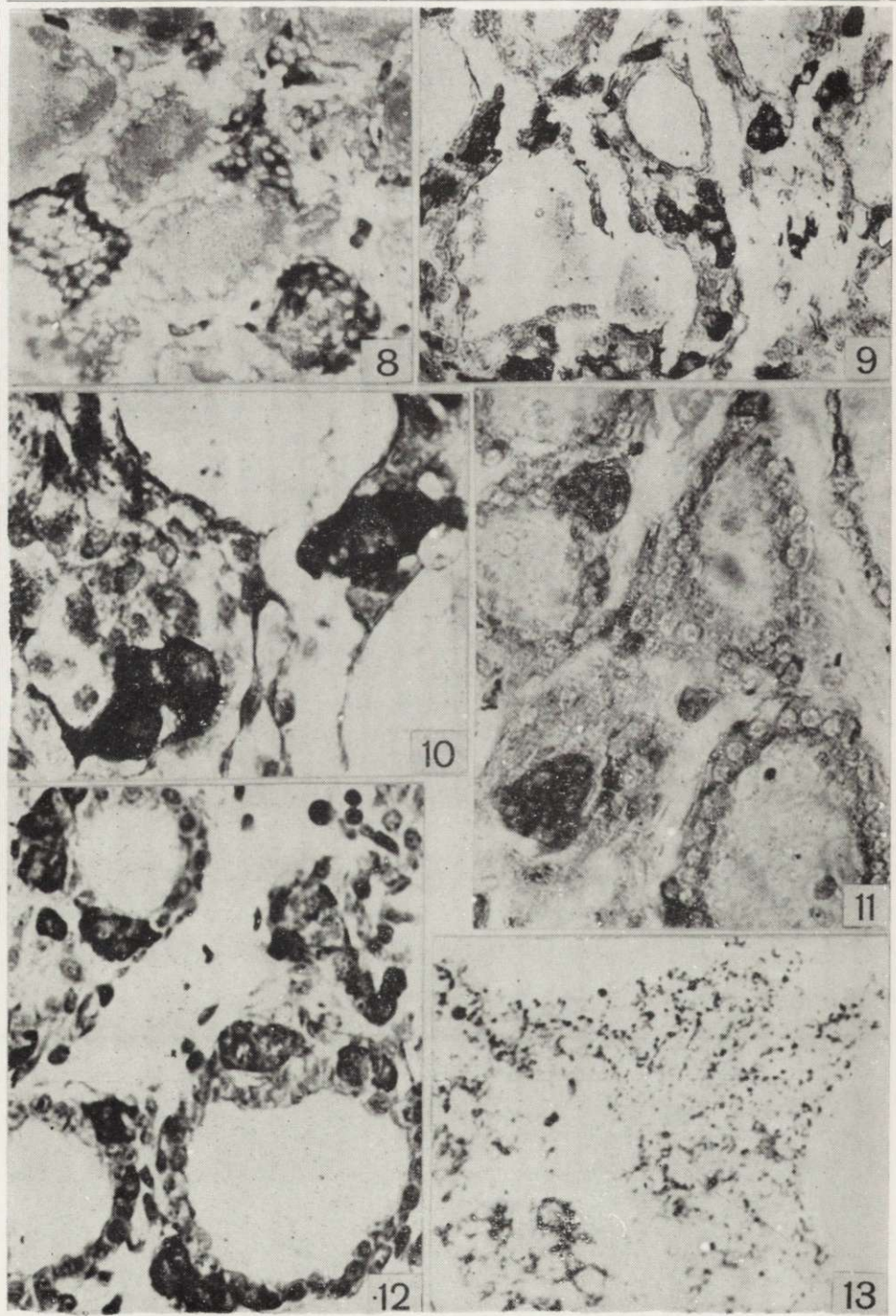
Plate VII.

- Fig. 14. Section of the thyroid of an adult guinea pig. Reaction to α -glycerophosphate dehydrogenase (α -GPD). The very low activity of this enzyme in the flat epithelium lining the follicles is remarkable. C cells are clearly differentiated owing to more intensive enzymatic activity. Magn. $\times 300$.
- Fig. 15. Section of the thyroid of an adult guinea pig. Reaction to α -glycerophosphate dehydrogenase in the follicle cells changes markedly with change in the height of the epithelium. Magn. $\times 750$.
- Fig. 16. Section of the thyroid of a young guinea pig. Reaction to α -glycerophosphate dehydrogenase (α -GPD). Magn. $\times 300$.
- Fig. 17. Section of the thyroid of a young guinea pig. Reaction to non-specific esterases (NE) with α -naphthol as substrate. Magn. $\times 300$.
- Fig. 18. Section of the thyroid of an adult guinea pig. Reaction to pseudocholesterases (PCh). Magn. $\times 150$.
- Fig. 19. Section of the thyroid of an adult guinea pig. Reaction to acid phosphatase (APh). Magn. $\times 300$.



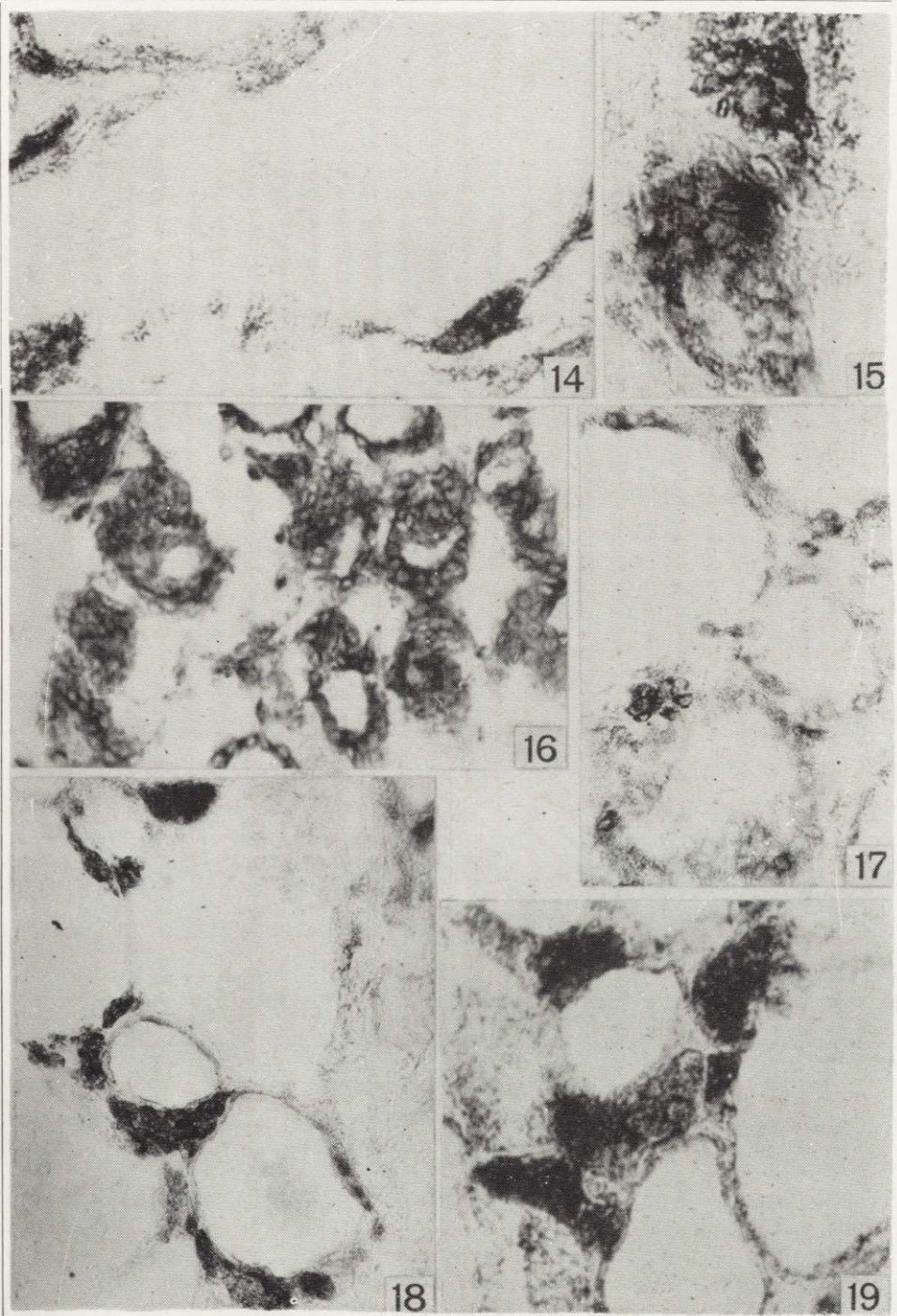
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