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The thyroid gland of *Callithrix jacchus* in organ culture

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Summary. Thyroid glands from 7 marmosets (*Callithrix jacchus*) of different age groups (newborn, 2 weeks, 2, 7, 8 and 11 months, and 8 years) were grown as organ culture according to Trowell at the medium/air interphase for 3 to 30 days. The morphology of thyroid tissue was well preserved until the end of the culture period. Necroses were only occasionally seen in connective tissue. In contrast to the in vivo situation, the number of lysosome-like inclusions changed considerably and cell-cell contacts became loose. After the addition of TSH the number of apical microvilli, apical granules and coated vesicles rose. The basal surface of follicle cells enlarged due to the formation of processes or ridges and invaginations. Mitosis could be observed. These findings show that, using the Trowell culture technique, thyroid tissue can be grown for up to 4 weeks without showing distinct changes in its morphology. Its responsiveness to the application of TSH is maintained in vitro. Hence, this technique is suitable also for long-term endocrinological, pharmacological and toxicological investigations.

Key words: Thyroid gland, Long-term organ culture, Follicle cells, TSH, Marmosets

Introduction

Numerous publications are available on the cultivation of thyroid glands (Ambesi-Impiombato and Coon, 1979). These reported mainly on the use of isolated follicle cells (Asakawa et al., 1992; Chabaud et al., 1992; Gruffat et al., 1992; Zurzolo et al., 1993) or isolated follicles (Nitsch and Wollman, 1980; Herzog and Miller, 1981; Miyagawa et al., 1983; Kitajima et al., 1985; Garbi et al., 1986; Toda and Sugihara, 1990; Sasaki et al., 1991; Bechtner et al., 1992; Gärtner, 1992; Many et al., 1992; Rousset et al., 1992). Experiments with epithelial cells plus matrix components or fibroblasts have revealed the importance of connective tissue (mesenchymo-epithelial interactions) for morphogenesis (Hilfer and Stern, 1971; Hilfer and Pakstis, 1977; Bell et al., 1984; Toda and Sugihara, 1990) as well as for the function of this gland. This influence of connective tissue is based on the chemical composition of the matrix and the binding of individual components to the cells, e.g. via integrins. Additionally, the physical properties of the matrix play an essential role (Karst and Merker, 1988). Moreover, the various products of mast cells (Catini and Legnaioli, 1992; De Forteza et al., 1992), the endothelium by means of endothelin secretion (Miyakawa et al., 1992; Eguchi et al., 1993), dendritic cells during autoimmunological processes (Mooij and Drexhage, 1992) and finally monocytes by producing several cytokines are able to influence follicle cells and their functions (Matsunaga et al., 1988; Nagataki and Eguchi, 1992). However, connective tissue is mostly removed during isolation of the cells or follicles prior to cultivation. Another disadvantage of in vitro models which require isolation of cells, is the limited cultivation period. Hence, they are less suited for long-term investigations, e.g. of the proliferation behaviour, which is important for numerous thyroid-influencing substances.

In contrast, using the organ culture technique, pieces of the thyroid gland are cultured without prior separation or isolation of its component, i.e. the ratio and morphological distribution of epithelium and connective tissue are normal. Under these conditions the function of the thyroid gland and its response to stimulating or inhibiting stimuli should be preserved and be better comparable to the in vivo situation. Using this technique the material can be grown for hours (Feeney and Wissig, 1971; Luttermann and Böcker, 1982; Remy et al., 1983; Pic et al., 1984) and days (Young and Baker, 1982; Asakawa et al., 1991; Takizawa et al., 1993), or up to 8 weeks (Bauer and Herzog, 1988). Especially the works of Bauer and Herzog (1988), which have demonstrated a long vitality and have been very well analyzed morphologically, appear to be of great interest for the in vitro experiments with thyroid tissue, although the required conditions (50% O_2, 5.6 mM D-glucose) aggravate the applicability to the in vivo situation. Floating cultures also involve some difficulties. We

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therefore tried to employ a modified organ culture technique, the so-called Trowell culture (Trowell, 1959) where organ pieces are grown under simple conditions on a filter at the medium/air interphase.

Reliable data have been obtained during the last few years on the morphology of the thyroid gland in vivo and the morphological substrate of its function (Herzog, 1984; Fujita, 1988; Ericson and Nilsson, 1992). Thus, the structure allows us to draw conclusions as to the function. Hence, a functional interpretation of the morphology of the thyroid gland should also be possible in organ cultures.

Pharmacological-toxicological research is facing the problem of species-specific effects of substances. Therefore, great efforts have been made to use primates as experimental animals. However, most primates show a difficult reproduction behaviour, require complicated maintenance conditions and additionally show other disadvantages (size, availability, etc.). In contrast, new-world monkeys (*Callithrix jacchus*) appear to be better suited for these purposes (Heger and Neubert, 1988; Neubert et al., 1992a,b). They can become pregnant twice a year (gestational period = 143 days), their adult weight is only 400 - 500 g, their maintenance is fairly easy and, given the appropriate surroundings and personnel, they can be tamed. In toxicological investigations they have shown reactions similar to those of man (Neubert et al., 1987, 1988). Since the number of available animals is small, in vitro methods using tissues from these primates are of special importance. Therefore the aim of this study was to test the possibility of performing Trowell organ cultures (Trowel, 1959) of the thyroid gland from *Callithrix jacchus* (marmoset), to draw conclusions from the morphology of this tissue to the functional behaviour under different in vitro conditions and to define the vitality of these cultures. Based on the obtained findings the utility of this in vitro model for endocrinological and toxicological research should be evaluated.

Materials and methods

Thyroid glands from marmosets (*Callithrix jacchus*) of our own breeding (Heger and Neubert, 1983; Neubert et al., 1988) served for the experiments. Table 1 shows the age groups used.

The thyroid glands were removed under Trapanal (Byk Gulden) anaesthesia. The animals were sacrificed by an overdosage of this substance at the end of anaesthesia. After disinfection of the cervical region with 70% alcohol the thyroid glands were removed under sterile conditions, placed into a Petri dish containing culture medium and cut. Up to 20 pieces can be obtained from the gland of a newborn animal.

Three tissue pieces were placed onto a membrane filter (5 x 20 mm, type SM 13307, Sartorius, Göttingen, Germany, pore size 0.2 µm), which rested at the medium/air interphase on a small bridge made from a stailess steel grid (5x20x2mm). Plastic petri dishes, diameter 35 mm, containing one grid, were filled with 2 ml growth medium. Complete medium changes were done twice weekly. Cultures were incubated at 37 °C in a humidified atmosphere with 5% CO_2 in air. Growth medium consisted of Dulbecco's modification of minimal essential medium or Ham's F-12 medium, 10% foetal calf serum, 100 IU penicillin/ml, 100 µg streptomycin/ml, 2 µg amphotericin B and 50 µm ascorbic acid/ml (Zimmermann et al., 1994).

After a culture period of 3 to 30 days (see Table 1) the tissue pieces determined for electron microscopy were fixed in Karnovsky's solution (3% glutaraldehyde + 3% paraformaldehyde in 0.1M phosphate buffer, pH 7.2). Post-fixation was done in 1% buffered OsO_4, which was followed by dehydration in the alcohol series and embedding in Epon. The sections were cut on an Ultracut (Leica), contrasted with uranyl acetate/lead citrate and examined under Zeiss electron microscopes EM 10 and EM 109. Thick sections (1 µm) were stained with Toluidine blue. For light microscopy the sections were fixed in 10% buffered formaldehyde, embedded in paraffin and stained with haematoxylin/eosine or according to the Azan method.

Thyroid-stimulating hormone (TSH; Sigma, 20 mIU/ml) was added either once on day 2 of the culture period or twice on days 2 and 9. The material was further cultivated and fixed on day 11 or 14 (Table 1).

Results

Epithelium

The basic structure of the thyroid gland consisting of

Table 1. Thyroid organ culture.

AGE OF THE ANIMALS	DURATION OF CULTIVATION	TSH-TREATMENT
Newborn	3 days	1 x
	11 days	2 x
	14 days	2 x
2 weeks	3 days	1 x
	11 days	2 x
2 months	14 days	-
	18 days	-
	30 days	-
7 months	14 days	-
	18 days	-
	30 days	-
8 months	3 days	-
	6 days	-
	11 days	-
	14 days	-
	18 days	-
11 months	3 days	-
	6 days	-
	11 days	-
	14 days	-
	18 days	-
8 years	8 days	-
	14 days	1 x

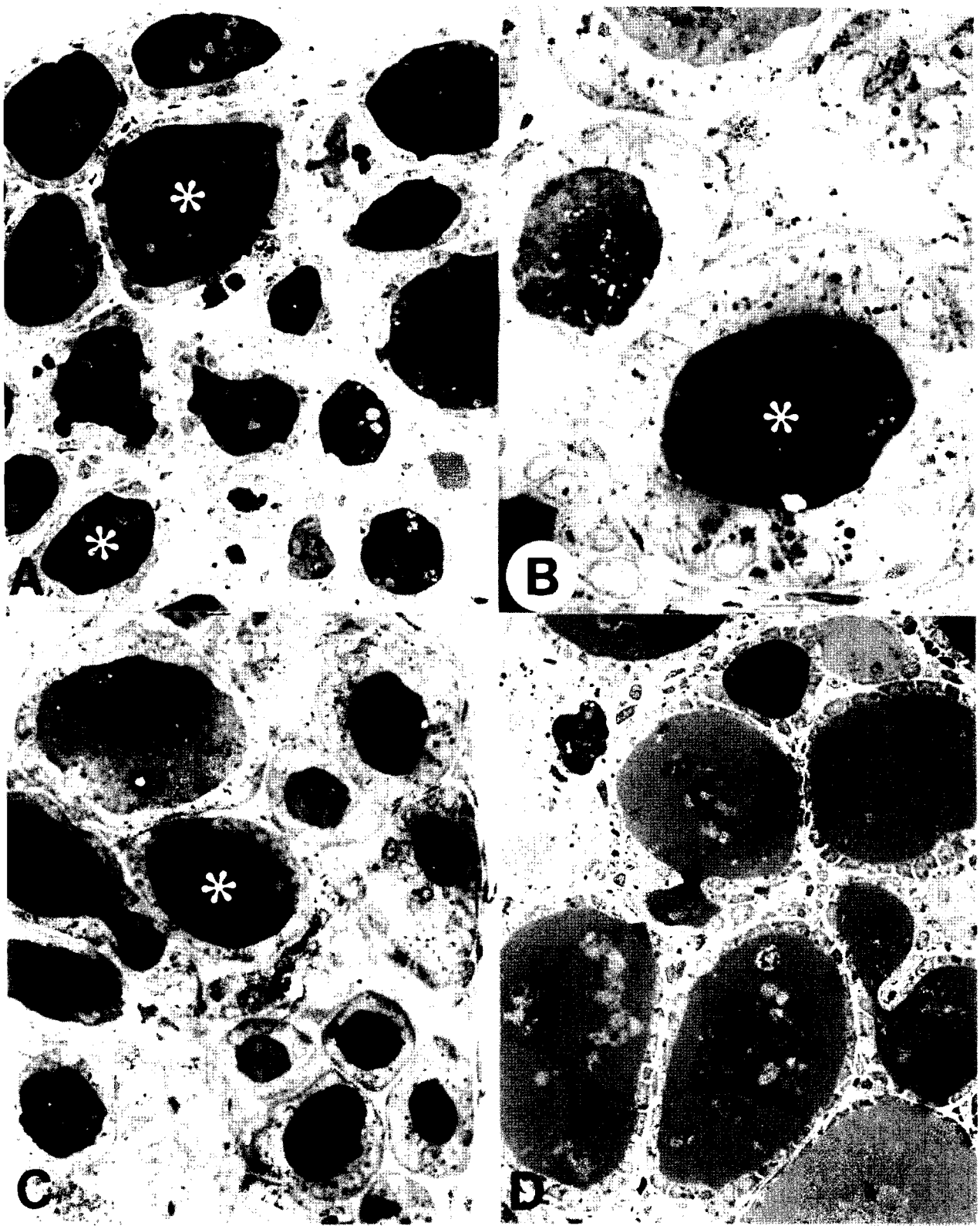


Fig. 1. Light micrograph of cultured thyroid glands from marmosets (semi-thin sections, Giemsa staining). **A.** Newborn animal, culture period - 3 days. x 400. **B.** Newborn animal, culture period - 11 days. x 1,000. **C.** 8-week-old animal, culture period - 30 days. x 400. **D.** Adult animal, culture period - 30 days. x 400. Follicles with darker colloid (*) or with lighter colloid (C).

follicles with colloid and connective tissue was maintained in organ culture even after as long a period as 30 days (Figs. 1, 6). Only connective tissue showed some necroses and cell debris. Surprisingly, the follicular epithelium did not exhibit necrotic cells. The age of the marmosets from which the thyroid pieces originated was still recognizable in the cultures. The thyroid glands of adult animals (8 years) also had very big follicles in vitro (up to 700 µm) with thin epithelium (Fig. 2D). The glands of newborns, however, lacked these large follicles. Small and medium-sized follicles with cuboid epithelium predominated (Figs. 1A,B, 4). In vivo thyroid glands from newborns occasionally revealed folliculogenesis processes, whereas in vitro such events (cell accumulations without follicular lumen) were no longer observed. Hence, folliculogenesis was completed in vitro. The fine structure of epithelial cells hardly changed in vitro as compared with the in vivo situation (compare with Maile, 1995). The number of epithelial microvilli greatly varied, whereas that of apical granules was low (Figs. 3, 4, 6A). The cytoplasm of numerous cells contained membrane-bordered, round to polygonal (sometimes up to 1 µm) electron-dense inclusions (Figs. 3B, 4, 6, 9C) which, according to other authors, represent lysosomes (Fujita, 1988). The larger inclusions of some cells contained membranous and granular structures as well as round, less electron-dense areas (Figs. 4A, 5, 6C), thus resembling residual bodies. However, epithelial cells and whole follicles existed which showed only few or no inclusions (Figs. 3A, 6A). This was mainly true of smaller follicles with cuboid epithelial cells. The rough endoplasmic reticulum was well developed, a Golgi apparatus of medium size was present and the oval mitochondria of the Crista type did

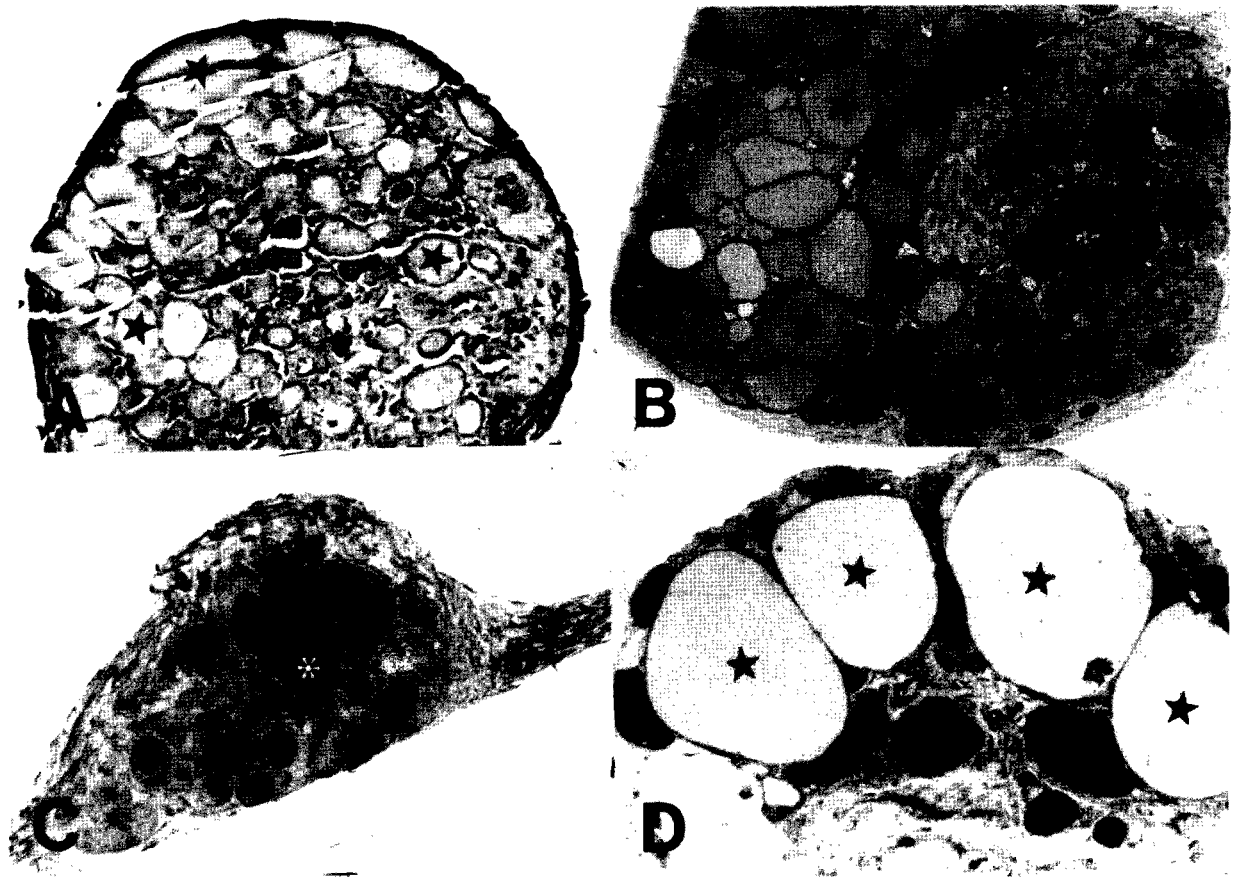
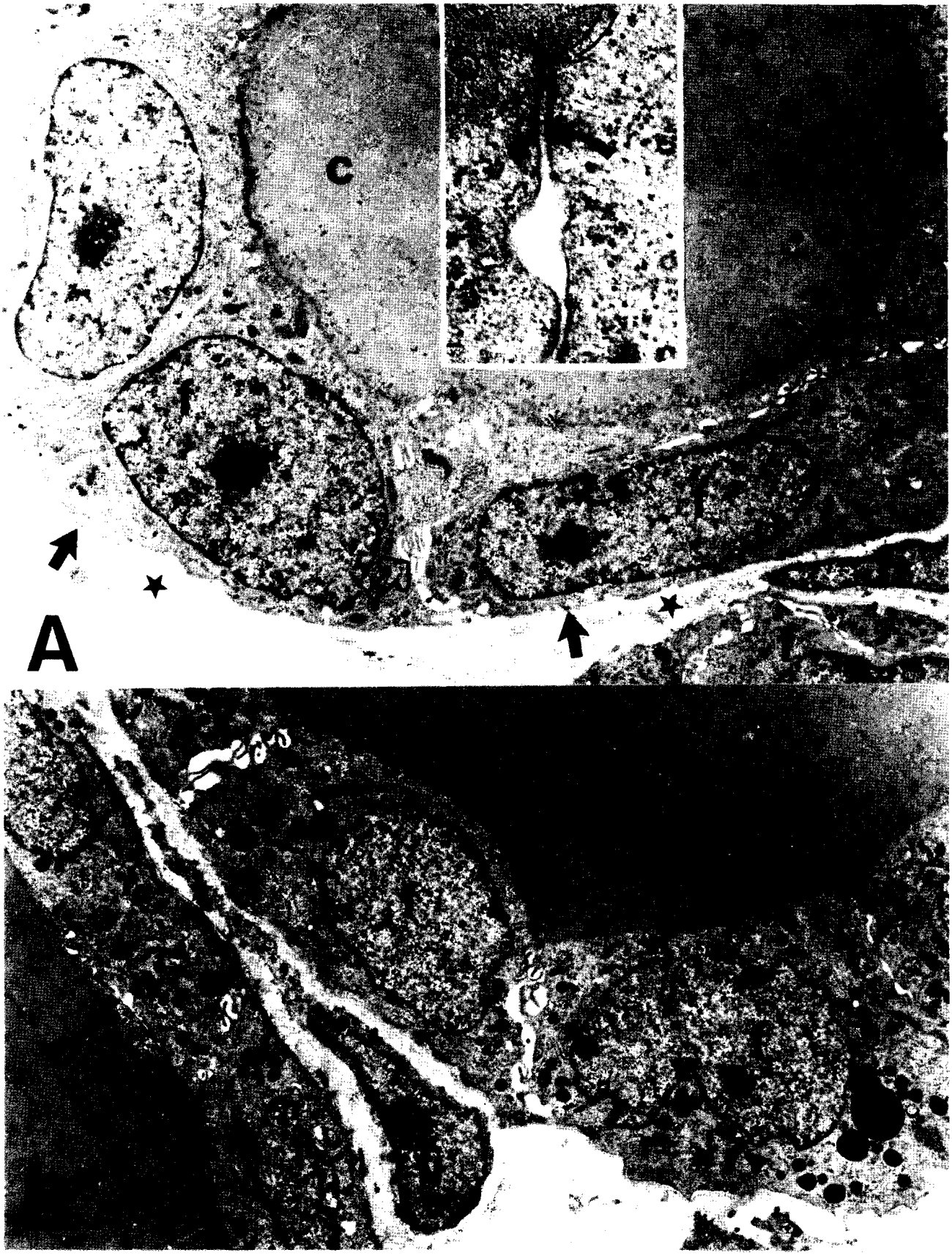


Fig. 2. Cultured thyroid glands of marmosets (semi-thin sections, Giemsa staining). **A.** Newborn animal, culture period - 11 days, TSH applied twice, follicle (*). x 120. **B.** 2-week-old animal, culture period - 11 days, TSH applied twice, follicle (*), C: cluster of c-cells. x 130. **C.** see Fig. 2 B. x 130. **D.** 8-year-old animal, culture period - 14 days. Large follicles with plate-like epithelium (*). x 130

Fig. 3. Thyroid gland of an 11-month-old marmoset after a 3-day culture period. **A.** Small follicles with cuboid to slightly flat epithelium (f). The cells do not contain any electron-dense inclusions. Smooth and regularly-running abluminal cell membrane (arrow). Intercellular spaces somewhat dilated, showing numerous microvilli (open arrows). fi: fibroblast-like cells with long processes (*). C: colloid. x 6,000. Insert: Apical contact area in the adluminal region showing Zonula adherens (open arrow) and normal (dark arrow) and wide intercellular space, but no tight or gap junctions. x 61,000. **B.** Follicular epithelial cells, but with numerous electron-dense granules of varying size, probably lysosomes. x 6,000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not show any alterations in vitro either (Figs. 3A, 4A).

However, a distinct difference existed between in vivo and in vitro as far as the cell contacts were concerned. In vitro, the lateral cell membranes moved apart and formed intercellular spaces of up to 1 µm (Figs. 3-8). Microvilli-like processes of neighbouring cells reached into these spaces without coming into contact with one another. They were stump-like or could reach a length of 1 µm. Their arrangement occasionally resembled loose interdigitations (Figs. 4, 5). These dilatations occurred independent of the age of the animals and the duration of cultivation. The apical contact region, which was orientated rectilinearly and radially, showed 1-2 small desmosomes and more towards the lumen a Zonula adherens with a submembranous densification consisting of fine filaments and a narrowing of the intercellular gaps to approximately 10 nm (Figs. 3, 4A, 9B). Tight or gap junctions were, in contrast to the in vivo situation, not demonstrable.

The contour of the basal cell membrane was, apart from some finger- or plate-like processes, smooth (Figs. 3, 6A). It was accompanied by a characteristic basal lamina (BL) consisting of a Lamina densa (50 nm) and a Lamina rara (30-40 nm). Occasionally the BL exhibited a multilayered arrangement. In these areas up to 6 *Laminae densae* were observed which ran more or less parallel at a distance of 20-100 nm (Fig. 6C). In other areas the Lamina densa homogeneously thickened to 500 nm. In both thickened areas the number of finger- or plate-like processes of the basal cell membrane was increased. But the length and width of the thickened segments appeared to be less pronounced than in vivo (see Maile and Merker, 1995). The pericapillary basal lamina could be missing in certain areas. This was especially the case when endothelial cells were transformed into fibroblast-like cells (see below). Clear-cut statements cannot be made on the behaviour of the basal lamina around small nerves since they apparently perished quickly after cutting the axons in vitro and were thus no longer demonstrable.

Interstitial space (connective tissue)

Analyzing electron microscopic pictures of connective tissue the well-preserved state of the cellular components even after a long cultivation period (30 days) was surprising, although cell debris also occurred. Their quantity differed from culture to culture and within one culture from area to area. They were not dependent on the duration of cultivation, i.e. such cell debris could be observed after 3 days as well as after 30 days in vitro. Necrosis probably did not proceed via the apoptosis mechanism, for nuclei and cytoplasm showed too few indications of densification or shrinkage. The cell organelles were often swollen and the membrane was fragmented (Figs. 3B, 5, 6A,B).

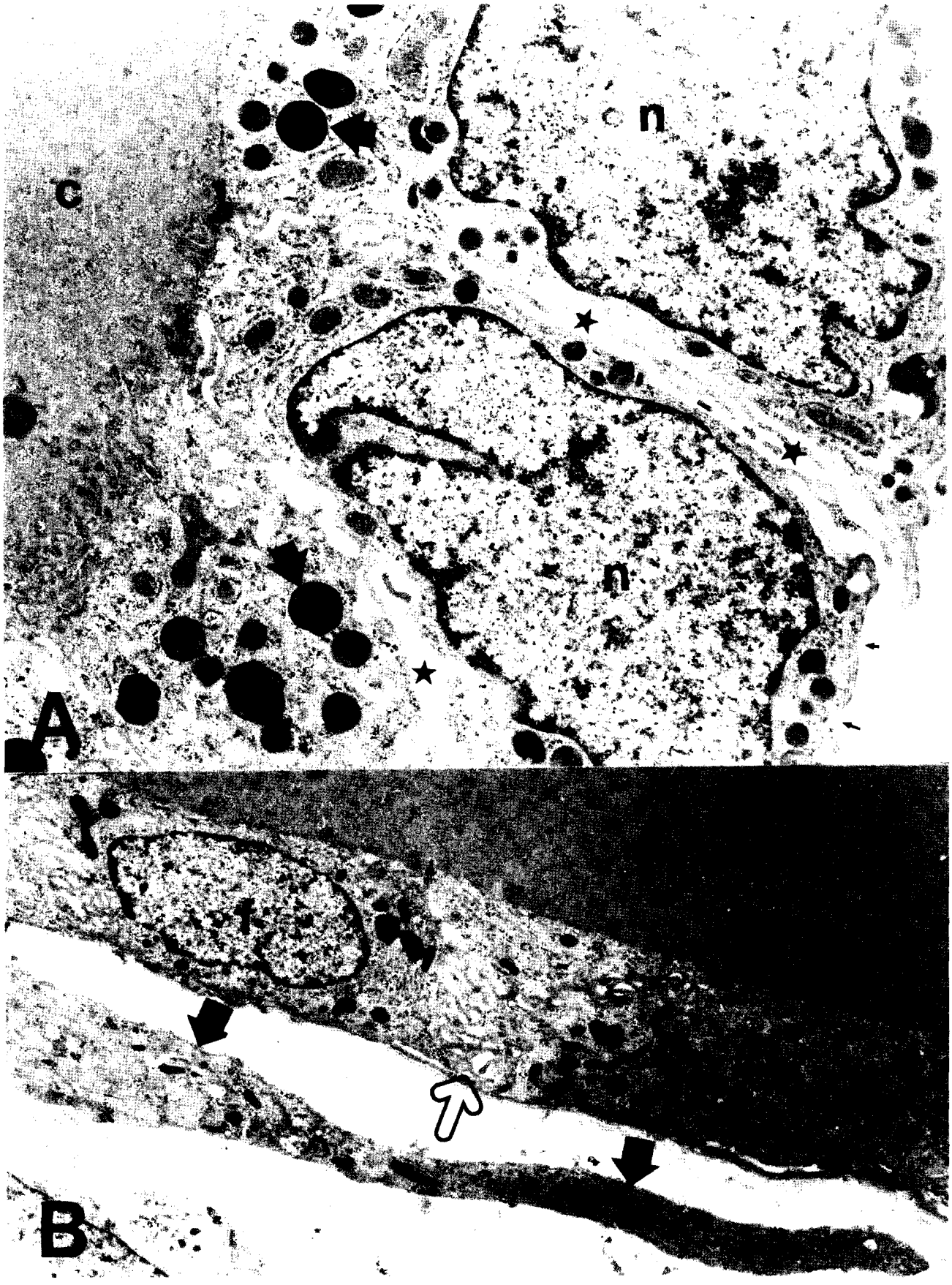
Numerous capillaries could clearly be identified up to a 30 days culture period (Fig. 6B). The endothelium was relatively thick (1-2µm) compared with that in vivo and pores were missing. The missing content led to a deformation accompanied by a narrowing of the lumen (Figs. 5, 6B, 8A). But indications of a disintegration of capillaries could also be demonstrated. The basal lamina disappeared, the endothelial cells became plumper and their processes extended into the neighbouring connective tissues. Finally, the contacts between the endothelial cells disappeared and they could no longer be clearly distinguished from fibroblast-like cells. The lumen of the capillaries often contained cell debris, occasionally also intact cells, mainly monocytes.

Lymph vessels, however, were no longer demonstrable. It cannot be stated with certainty whether some of the elongated cells with their processes of varying length and width (up to 0.8 µm) originated from endothelial cells of lymph vessels. It was not possible either to follow the fate of dendritic cells (Maile and Merker, 1995). They disappeared with their long thin processes under in vitro conditions. The plump, fibroblast-like cells with thicker, plate-like processes might also represent changed dendritic cells (Figs. 3-5).

Some of the bipolar or multipolar cells with a well-developed rough endoplasmic reticulum were probably genuine fibroblasts (Figs. 6A, 6B, 7D). In addition, round cells with a smooth surface and a few cell organelles with indented nucleus were observed. Their morphology suggests them to be monocytes (Figs. 6, 7A). All transitional stages from such cells to the typical large macrophages (Fig. 7B) with numerous inclusions and an irregular surface occurred. Finally, the mast cells must be mentioned, which represent a typical component of thyroid connective tissue (Fig. 7C). In vitro, all of them were at the stage of degranulation, thus their granules were ballooned, partly empty or contained only loosened remainders of their original content. Small round cells with relatively large round nucleus and narrow cytoplasmic rim were only rarely seen. They are likely to represent lymphocytes.

The morphology, quantity and distribution of the structures of the intercellular space largely corresponded to the in vivo situation. Collagenous, cross-striated fibrils (20-30 nm) were observed which ran singly and irregularly or in bundles. Additionally, filaments without cross-striation (approx. 10 nm) could be demonstrated which also ran singly and irregularly or in bundles (Figs. 3, 6, 7).

Fig. 4. Thyroid gland of a newborn marmoset after an 11-day culture period. **A.** Indications of highly prismatic epithelial cells with electron-dense inclusions (arrow). Between cells, dilated spaces (*) with numerous microvilli. n: nuclei of follicular cells; c: colloid; small arrow: lamina densa; arrowhead: junctional complex. x 15,000. **B.** Slightly flatter follicular epithelial cells (f). Dilated intercellular spaces with numerous microvilli (open arrow). Very long cell process in connective tissue (dark arrow). C: colloid. x 6,000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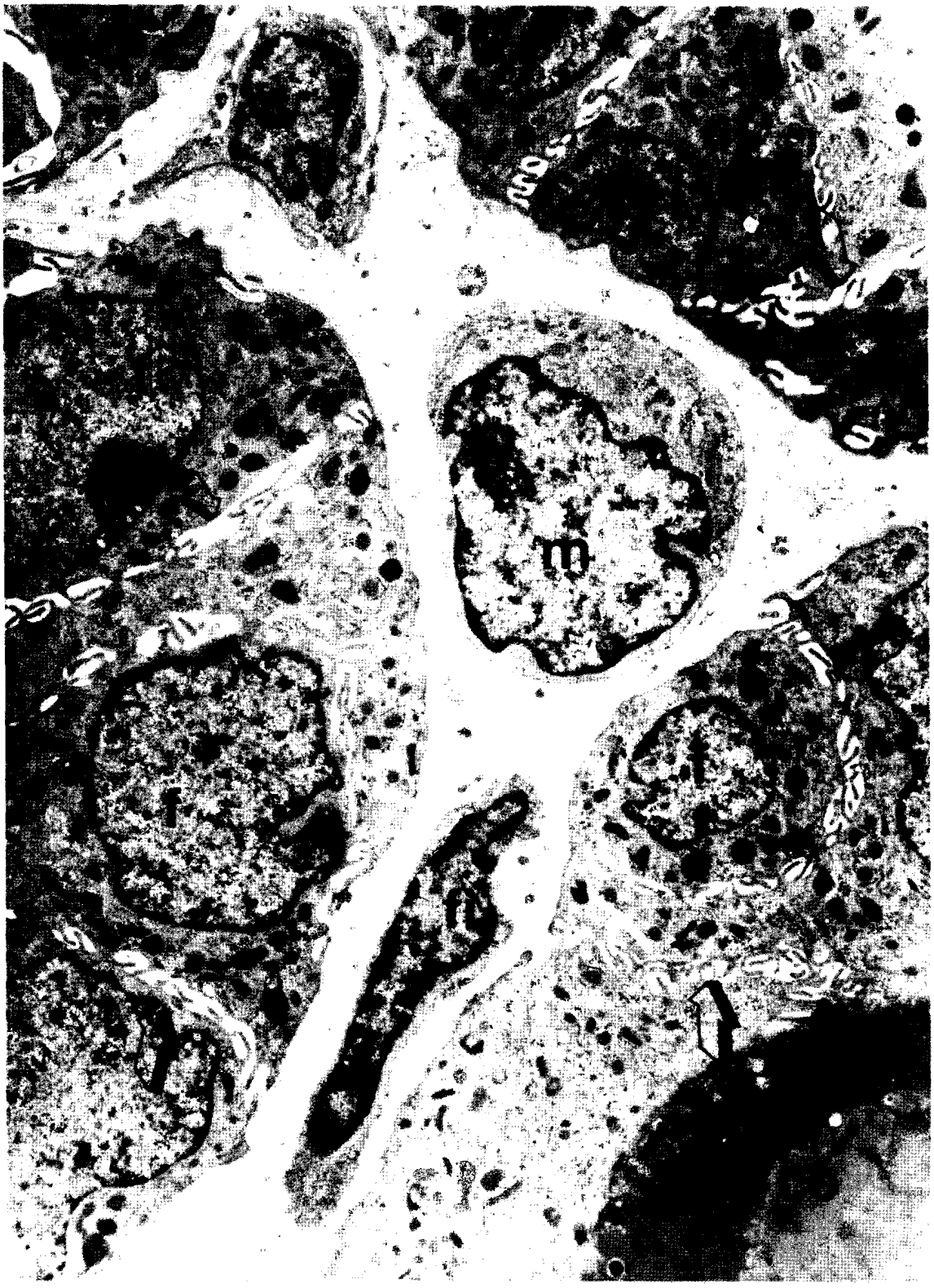
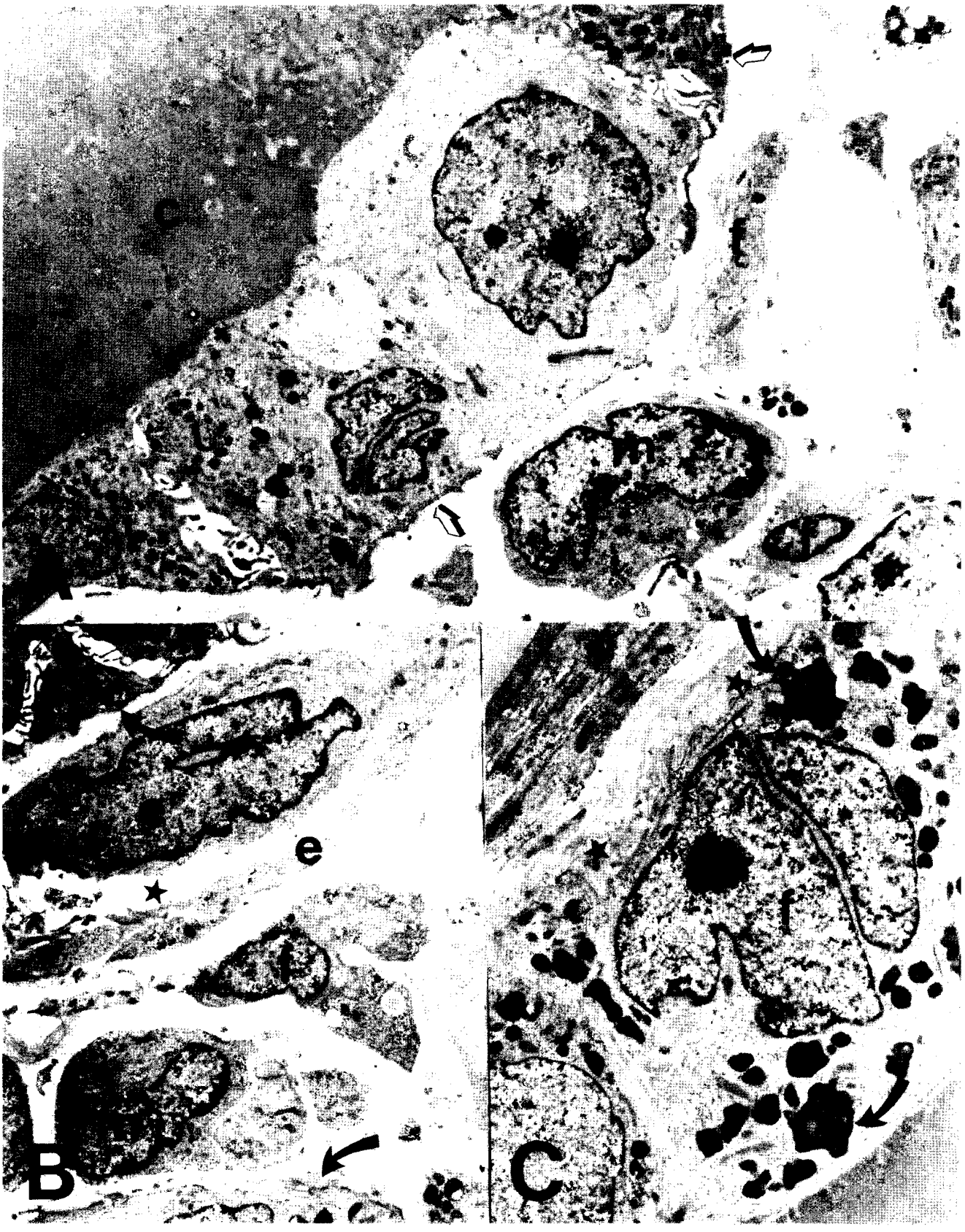


Fig. 5. Thyroid gland of an 8-month-old marmoset after a 14-day culture period. Cuboid epithelial cells (f) with varying amount of electron-dense inclusions (lysosomes). Dilated intercellular spaces with numerous microvilli (open arrow). Intercellular space showing collapsed capillary (c) with well-preserved endothelial cells, a monocyte-like cell (m) and a fibroblast (fi). CO: colloid. x 9,600

Fig. 6. Thyroid gland of a 2-month-old marmoset after a 30-day culture period. **A.** Follicular epithelial cells with and without (*) electron-dense inclusions. Well-preserved Lamina densa (open arrow) and relatively smooth contour of the abluminal cell membrane. Interstitial space showing monocyte (m), fibrocyte and cell processes (f). c: colloid. x 6,000. **B.** Interstitial space between two follicles (arrow) with monocyte (m), fibrocyte (f) and capillary with residual lumen (*) and well-preserved endothelial cells (e). x 6,000. **C.** Follicular epithelium (f) with electron-dense inclusions. Large inclusions with irregular contour containing round lighter areas (arrows). Multi-layered basal lamina (*). x 9,500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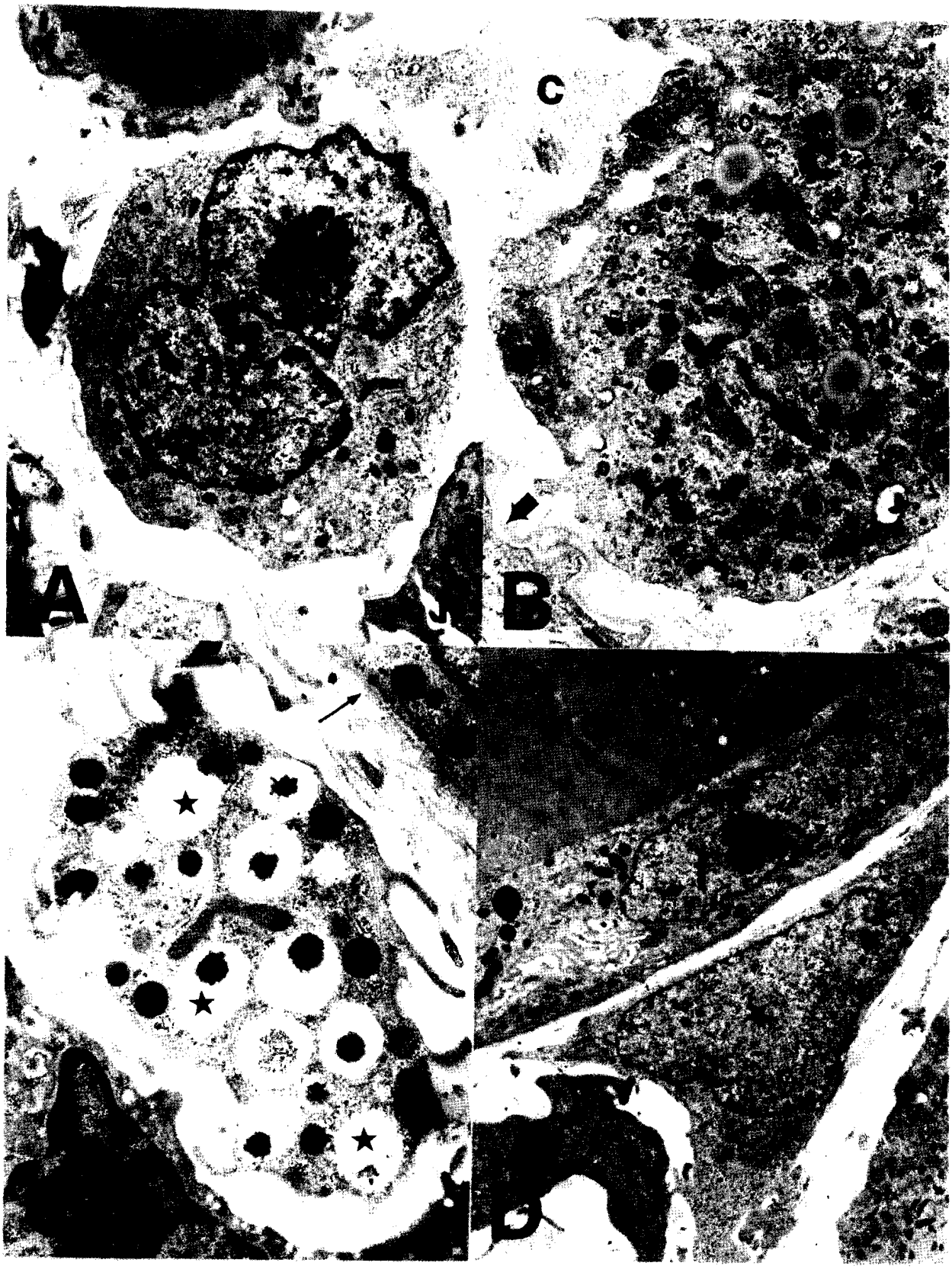


Fig. 7. Thyroid gland of an 11-month-old marmoset after an 11-day culture period. **A.** Monocyte with indented nucleus (n) and a small follicle in the vicinity (f). x 10,000. **B.** Macrophage (m) with numerous electron-dense inclusions and cell organelles next to a follicle (arrow) and a collagenous fibrillar bundle (c). x 20,000. **C.** Section of a mast cell with typical loosened or even empty vacuoles (*) indicating degranulation. In the vicinity, a fibrocyte (f) and a follicle (arrow). **D.** Fibroblast-like cell (*) in the vicinity of a follicle. f: follicular epithelial cell. C: colloid. x 6,500

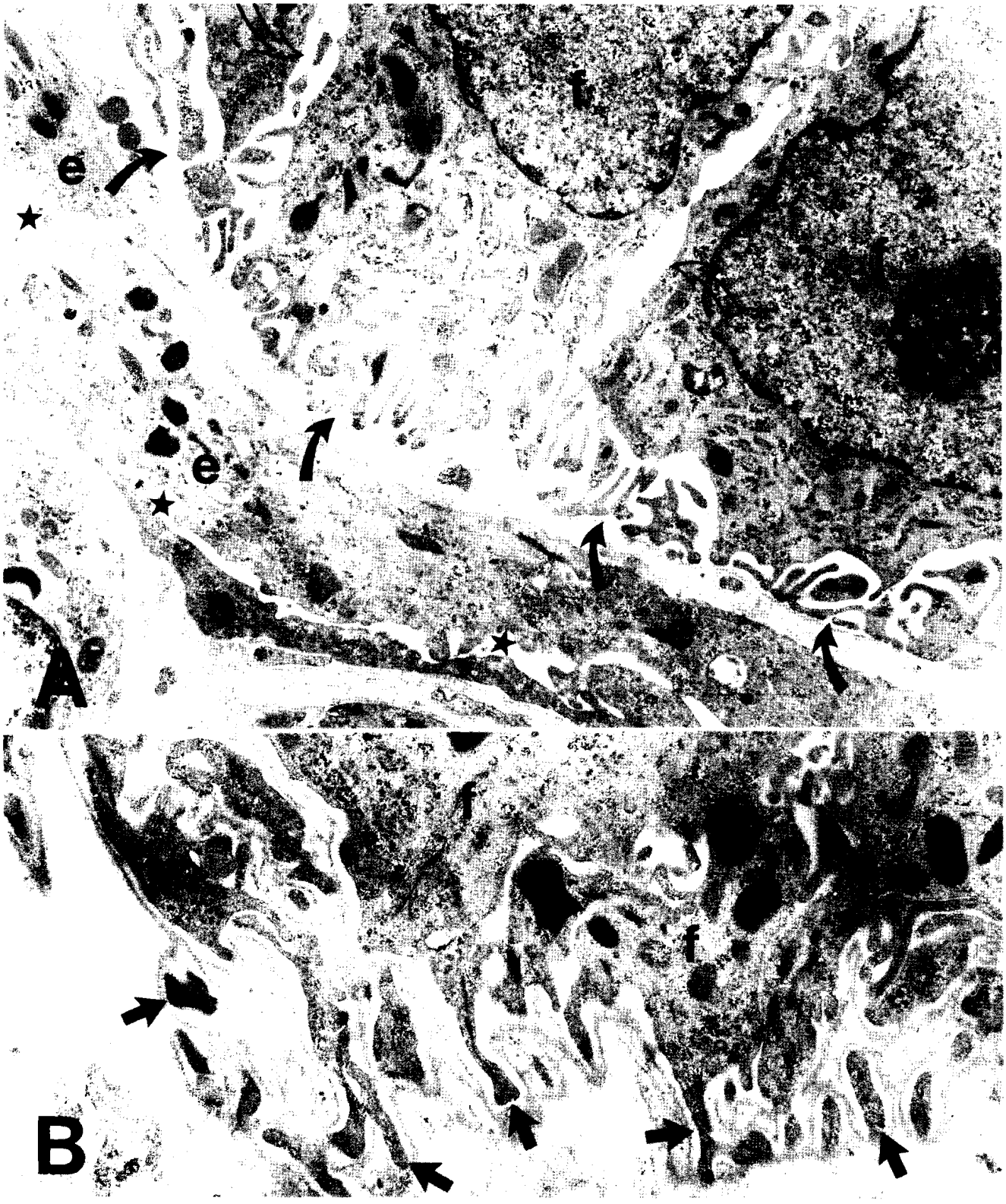


Fig. 8. Thyroid gland of a 2-week-old marmoset after an 11-day culture period; treated with 20 mIU/ml TSH on days 2 and 9. Irregular contour of the abluminal cell membrane (dark arrow) and dilated intercellular spaces (open arrow). In the vicinity, a collapsed capillary with residual lumen (*) and well-preserved endothelial cells (e). f: follicular epithelial cells. x 15,000. **B.** Thyroid gland of an 8-year-old marmoset after a 14-day culture period; treated with 20 mIU/ml TSH on day 3. Heavily indented abluminal surface of follicular epithelial cells (f) with numerous processes (arrow). x 25,000

TSH-Treatment

Although the protocol of TSH-application was obviously not optimal (only once or twice during a culture period of varying length), morphological differences were observed in the treated follicles as compared with the untreated material (Figs. 8-10). These differences included the contour of the basal cell membrane which was very irregular, showing numerous processes and indentations; smooth segments often alternated with very irregular segments. The dilatations of the lateral intercellular spaces with their microvilli were even more pronounced (Fig. 8A).

The behaviour of the apical microvilli had clearly changed. After TSH-treatment they increased in number and length (Fig. 9A,B). The number of apical granules and coated vesicles had also increased (Fig. 10B). Some follicular epithelial cells contained more lysosomes, others lacked these inclusions (Figs. 9C, 10A). Hence, the morphological picture greatly varied. Intracellular structures occasionally occurred which could not be observed in untreated material: large (up to 4 µm) homogeneous inclusions which were obviously phagocytosed out of the colloid, and cavities (up to 5 µm; Fig. 10A) from whose bordering membranes microvilli extended into the lumen. Occasionally, several such cavities occurred in one cell. The appearance of mitoses after TSH treatment was striking. Without treatment they were only very rarely to be seen. The other cell organelles and connective tissue of thyroid glands treated with TSH in vitro did not exhibit any differences.

Discussion

The structure of cultured thyroid pieces had not changed principally, as compared with the in vivo situation. Even the initial situation where we observed large follicles and a thin epithelium in adult animals and mainly smaller follicles with cuboid epithelium in young or newborn animals could still be seen in vitro. Also the fine-structural features of epithelial cells with their polar structure, electron-dense inclusions and the other cell organelles were well demonstrable, although single cells or whole follicles occurred which did not contain any inclusions. Assuming that these inclusions are of lysosomal nature (Wollman et al., 1964; Wetzel et al., 1965; Seljelid, 1965, 1967; Uchiyama et al., 1989; Kostrouch et al., 1993) and are formed during the resorption of the colloid, their absence might indicate a low resorption as well as a quick degradation without formation of residual bodies. Cells shortly before, during, and after mitosis did not have any inclusions either. But the number of such cells is obviously very small.

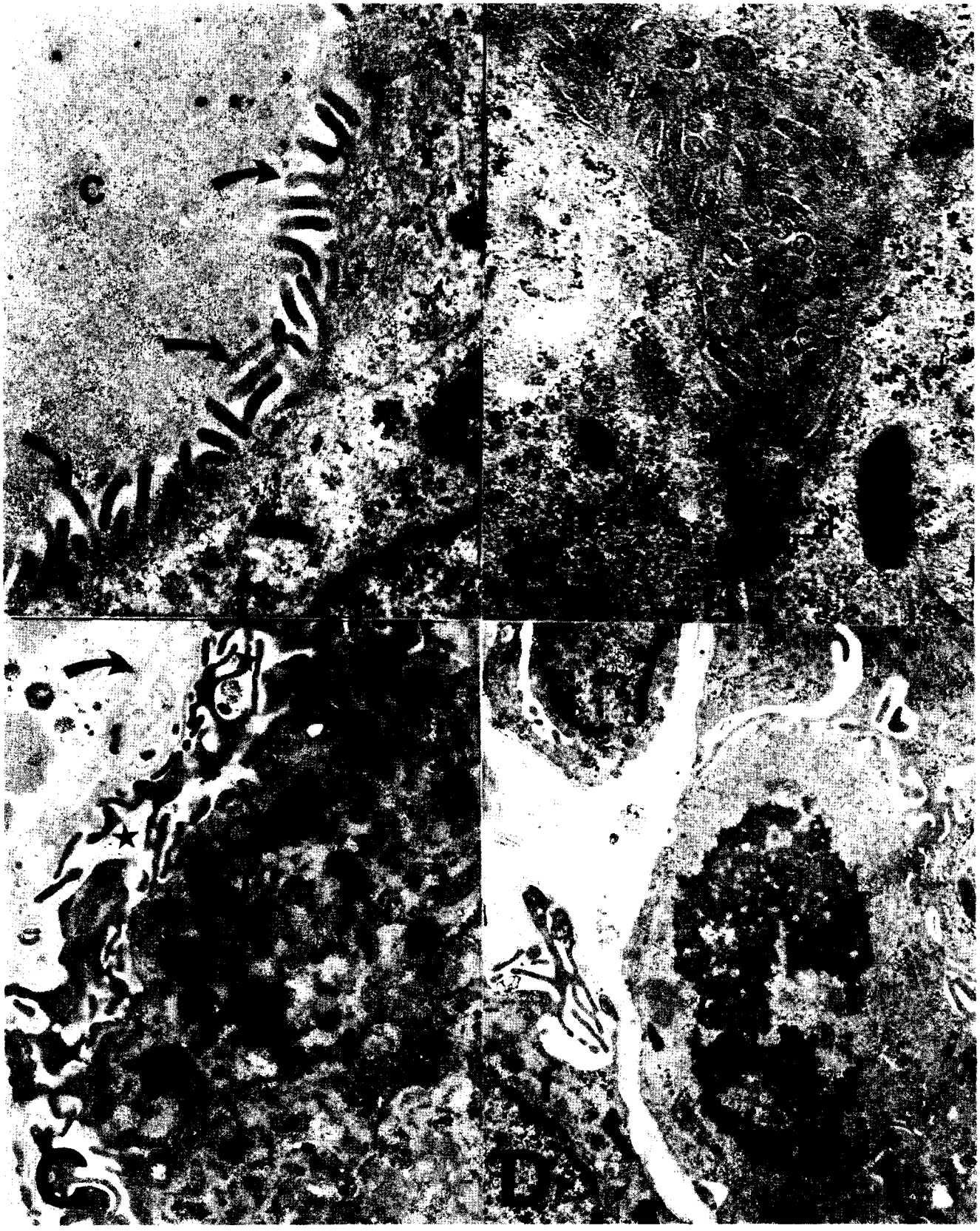
The continuation of a well-preserved state even in long-term cultures (30 days) was surprising. Once the cultures had adapted themselves to the in vitro conditions, the time factor apparently did not play such a great role. Investigations performed by Luttermann and Böcker (1982) according to the Trowell method revealed degenerative changes as early as after a 5-hour culture period. These differences to our findings may be due to different sizes of the tissue pieces (up to 2 µm in their material and approx. 0.3 mm in our material). Bauer and Herzog (1988) studied thyroid tissue pieces of 0.5 to 0.9 mm in floating culture and also observed necrotic changes when the O_2-concentration in the incubator was less than 50%.

A difference in the epithelial region between in vivo and in vitro was due to the widening of the intercellular gap accompanied by a pronounced process formation of the lateral cell membranes. Similar intercellular spaces can also be observed in cultured follicles (Chambard et al., 1981; Kitajima et al., 1985; Westermark et al., 1986). Since the supply of the epithelium after cessation of the circulation is probably no longer optimal, easier accessibility of the cells via a widening of the intercellular gap and enlargement of the surface due to process formation would be quite feasible.

In contrast to the epithelium, connective tissue showed some cell debris. Fragmentation of the cell membrane, swelling of the cytoplasm and membrane-bordered cell organelles speak against an apoptotic necrosis mechanism. Hence, some connective tissue cells are obviously more sensitive to in vitro conditions than the epithelium. Nevertheless, a major proportion of connective tissue cells survived in good state. Also the functionless capillaries could clearly be identified morphologically after a 30-day culture period. Connective tissue contained more monocytes than in vivo. Since resupply from blood or bone marrow is not possible, re-differentiation of the cells which originated from monocytes must be assumed: macrophages and dendritic cells. Both cell types occur in thyroid tissue of marmosets in vivo (Maile and Merker, 1995).

The good state of preservation of follicular epithelium as well as connective tissue confirm proper functioning of thyroid pieces also under organ culture conditions. This concept is corroborated by the response even of long-term cultures to TSH-treatment. Although the treatment schedule was not optimal (application once or twice), morphological effects could be observed as long as 3 days after application of the hormone. Increase and elongation of apical microvilli, increase in the surface of the basal cell membrane and increase in apical

Fig. 9. Thyroid gland of a newborn marmoset after an 11-day culture period; treated with 20 mIU/ml TSH on days 2 and 9. **A.** Increase and elongation of adluminal microvilli (arrow). Apical cytoplasm showing fine-filamentous network (small arrows) and microtubuli (arrowhead). c: colloid. x 35,000. **B.** Residual lumen of a follicle without colloid, but with numerous microvilli (*). The apical region of two neighbouring epithelial cells showing a junctional complex (arrow). x 49,000. **C.** Basal part of an epithelial cell (f) with heavily indented surface (*), very heterogeneous inclusions with round lighter areas (open arrows) and multilayered basal lamina (bent arrow). x 15,000. **D.** Epithelial cell in telophase (t). f: section of a follicle; c: capillary. x 6,000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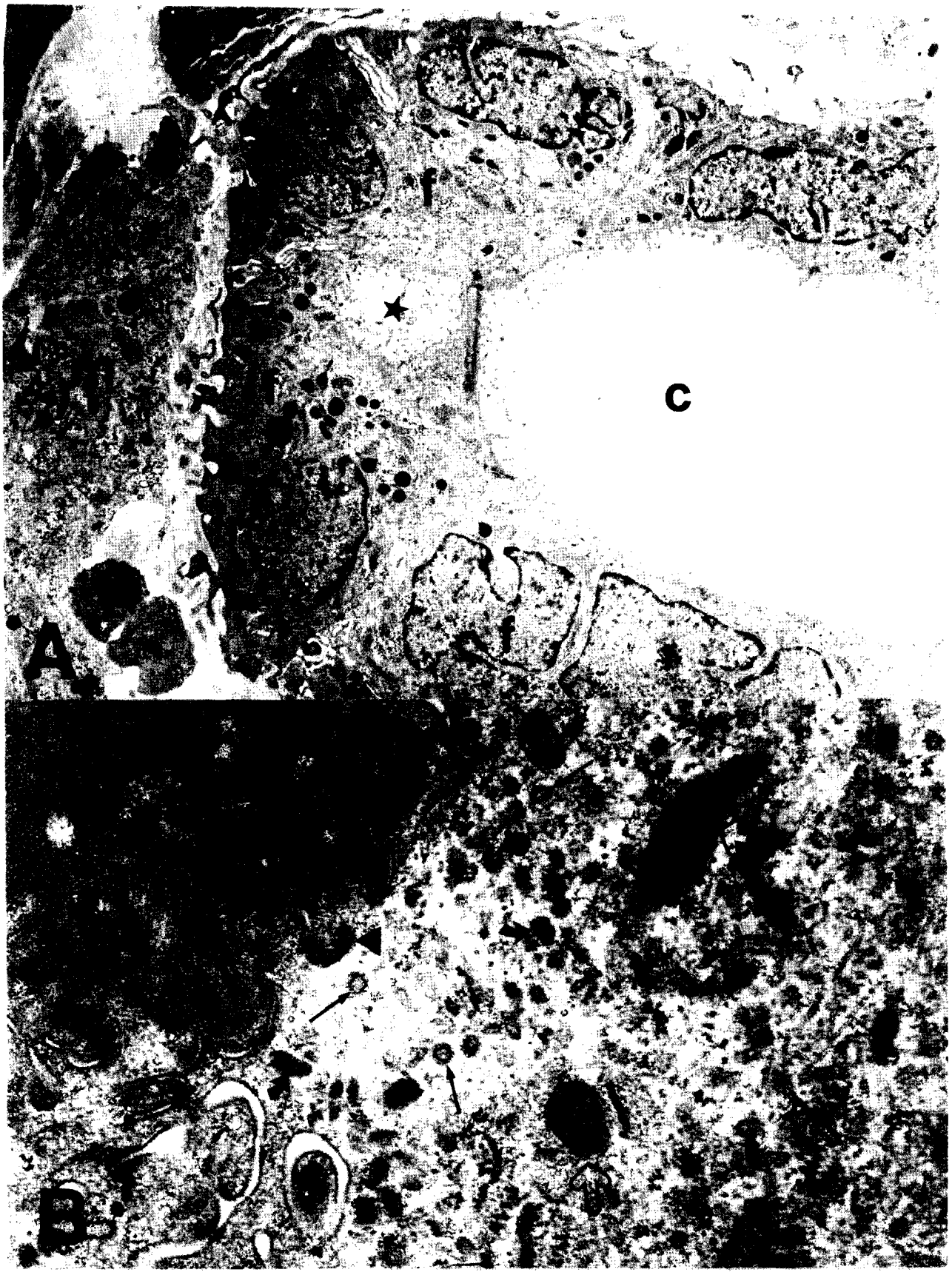

Fig. 10. Thyroid gland of a newborn marmoset after an 11-day culture period; treated with 20 mIU/ml TSH on days 2 and 9. **A.** Small follicular lumen (*) in an epithelial cell. c: colloid; f: follicular epithelium; m: macrophage. x 6,000. **B.** Apical part of a follicular epithelial cell with coated vesicles (arrow), coated pits (arrowhead) and typical granules (bent arrows), lysosomes (open arrow). * : numerous cell processes; c: colloid. x 61,000

granules and coated vesicles point to an enhanced activity. It cannot be decided using merely morphological means whether the enlargement of the basal surface implies an increase in TSH-receptors which are located on the basal cell membrane (Mizukami et al., 1994).

The good morphological and possibly also functional state of the cultured thyroid pieces probably also requires the presence of special factors in addition to the basic supply. It is known, for example, that secretion products of mast cells, endothelin from endothelial cells and cytokines from monocytes regulate function and behaviour of the thyroid gland (Sato et al., 1990; Catini and Legnaioli, 1992; Jackson et al., 1992; Eguchi et al., 1993; Oka et al., 1993). These three cell types occur at a sufficient amount also in vitro so that the existence of these substances can be taken for granted.

The good state of preservation of thyroid pieces under the described in vitro conditions and their response to TSH suggest an experimental use of these cultures. Especially the possibility of long-term cultivation renders this technique very useful. Thus, the large group of substances could be tested whose effects are due to an increase in the rate of proliferation. Moreover, it could be ascertained whether an effect of the thyroid gland is direct or indirect, e.g. via stimulation of TSH-secretion.

The described culture technique has additional advantages as compared with the other in vitro methods. A number of preparation steps can be omitted which aggravate such experiments or lead to a less satisfactory reproducibility, e.g. isolation, centrifugation, cell counting, or coating. None of our cultures was a failure in this respect.

Finally, another advantage of the organ culture technique should be pointed out. Twenty pieces could be obtained from the thyroid gland of one newborn primate. Hence, it is possible to perform large experimental series and statistical measurements using only a few glands. This allows us to drastically reduce the number of experimental animals, which is of special importance in the case of primates.

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