MATURATION OF THE RAT FETAL THYROID

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ABSTRACT

Maturation of the rat fetal thyroid was studied with the aid of I^{131} and of fluorescence and electron microscopy. The I^{131} concentration of the fetal gland increased exponentially from day 17 to day 20 of gestation and was related to the weight of the fetus (and presumably the weight of the thyroid) and also to the quantity of I^{131} accumulated by the fetus. In the 17-day gland, thyroglobulin or immunologically similar material was sparsely present in the incipient lumens of some cell clusters. With maturation, this material increased and was also observed within follicular cells on days 18 to 19 of gestation. On day 20, the specifically reacting material was present in the follicular lumens and was absent from the cytoplasm of follicular epithelium. Ultrastructurally, the earliest thyroid cells examined were replete with all the organelles found in the more mature epithelium. No direct correlation could be made between the cytoplasmic structures and the presence of thyroglobulin, although the granular endoplasmic reticulum was most likely the organelle responsible for synthesis of thyroglobulin. Thyroglobulin or a precursor was found in fetal thyroid cells before measurable quantities of I^{131} were concentrated and before cytoplasmic droplets appeared.

Between day 18 and day 19 of gestation, the fetal thyroid of the rat acquires the capacity to concentrate easily measurable quantities of I^{131} (1). Based on this property of isotope accumulation by the fetal thyroid, and with the aid of electron and fluorescence microscopy, the following study was designed to seek answers to three questions: First, does an intracellular organelle appear or become singularly prominent at the time the thyroid gland begins to trap iodide in high concentration? Second, during the maturation of the fetal thyroid, is it possible to localize the site of synthesis of specific thyroid protein, thyroglobulin? Third, is thyroglobulin or a precursor produced before the advent of iodide concentration by the gland?

MATERIALS AND METHODS

Pregnant rats (Holtzman) with known mating times were injected subcutaneously on the 16th to 19th day of gestation[1] with 50 to 60 μc of I^{131}. 24 hours later the fetuses were removed from their etherized dams and their thyroids were visualized under a stereomicroscope. The glands to be processed for electron microscopy were flooded with 2 per cent osmium tetroxide *in situ* as quickly as possible after removal of the fetus from the uterus, usually within less than 1 minute. After freeing from the trachea, they were placed in 1 per cent buffered osmium tetroxide containing sucrose for 1 to 2 hours and then processed for Vestopal W embedding. Other fetal glands were embedded in a small piece of maternal

[1] 24 hours after a vaginal smear positive for sperm was counted as 1 day of gestation.

TABLE I

Summary of Data on Rat Fetuses Removed from Dams Injected with I^{131} 24 Hours before Autopsy

	Day at time of autopsy			
	17	18	19	20
Body weight (gm)	0.8 ± 0.012* (42)‡	1.2 ± 0.03 (52)	2.0 ± 0.34 (41)	3.4 ± 0.004 (33)
Thyroidal radioactivity (CPM)	0 (43)–41 (16)	0 (19)–220 (29)	2327 (49)	22,061 (43)
Fetal radioactivity without thyroid (CPM)	483 (57)	1920 (48)	6322 (60)	21,376 (40)
Number of dams	17	10	12	8

* ± standard error of mean.

‡ Figures in parentheses show the number of fetuses used to determine each result.

brain and after counting for radioactivity were quickly frozen in liquid nitrogen and kept frozen at −30°C until sectioned for fluorescence microscopy. Maternal brain was employed as support for the minute glands to allow for better frozen sectioning. Still other fetal glands were extirpated and fixed in Bouin's solution for light microscopy.

All fetal thyroids were counted in a scintillation counter with a sensitivity of 1×10^6 CPM/μc of I^{131}. The radioactivity of those embedded in brain was determined by subtracting the counts per minute of a fragment of brain tissue from the same dam, equivalent in size and shape to the piece used to support the thyroids. The radioactivity in the fetuses, with or without their thyroids, was determined in a GM well counter with a sensitivity of 6.0×10^4 counts per minute.

For the fluorescent antibody technique, rabbit antiserum was harvested following multiple intradermal and subcutaneous injections of a bovine thyroglobulin (Tg)[2] in complete Freund's adjuvant. The globulin fraction of this serum was obtained by precipitation with half-saturated $(NH_4)_2SO_4$ and was conjugated to fluorescein-isothiocyanate. This conjugated fluorescent anti-Tg (Fl-anti-Tg) was used as an immunohistochemical stain in appropriate tissue sections to detect Tg. The methods of conjugation, fluorescent staining, and fluorescence microscopy were similar to those previously described (2, 3). In Ouchterlony agar plates, the Fl-anti-Tg produced a single heavy line of precipitation with the original bovine antigen, a double line with crude bovine aqueous thyroid extract, and a single band against rat and human aqueous thyroid extracts. The lines of precipitation of the extracts from the three species exhibited reactions of partial identity with

[2] We are grateful to Dr. J. Wolff, of the National Institute of Arthritis and Metabolic Diseases, United States Public Health Service, for generously giving us purified bovine thyroglobin.

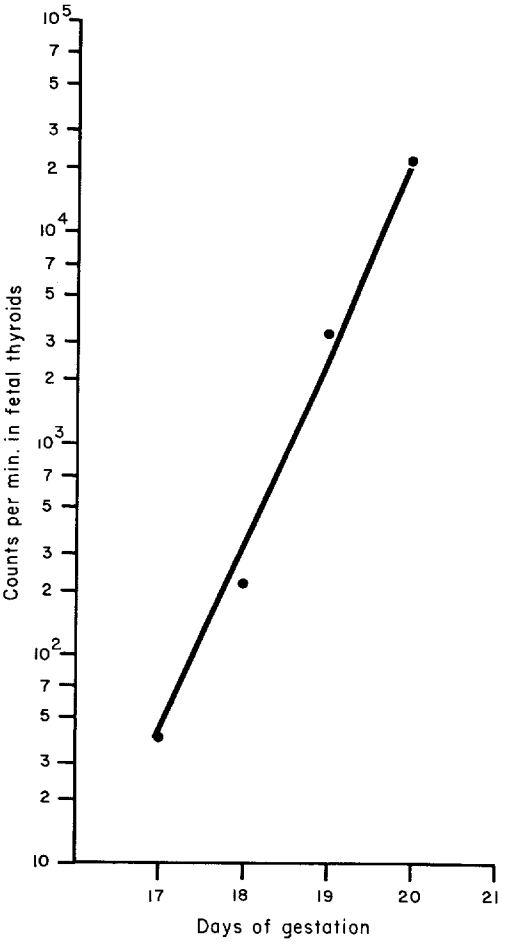


FIGURE 1

Semi-log plot of radioactivity in the fetal thyroid against gestation time. Each point represents the mean of the numbers of animals shown in Table I.

one another. When applied to sections of adult human and rat thyroids, the Fl-anti-Tg stained only colloid, while in sections of beef thyroid it stained both colloid and presumably the cytoplasm of follicular cells. As controls, both fetal and adult thyroids were stained with fluorescent unrelated antibodies, which yielded negative results. Also, the specific staining was inhibited when non-fluorescent anti-Tg was applied to the sections prior to use of Fl-anti-Tg, indicating the immunologic specificity of the positive results.

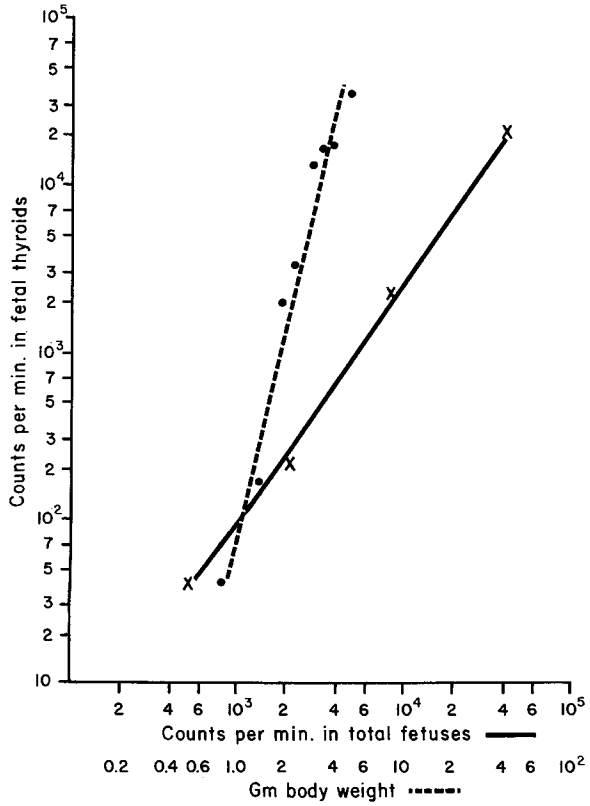


FIGURE 2

Log-log plot of radioactivity in the fetal thyroid against radioactivity in the fetus with thyroid and against body weight.

RESULTS

The isotope data are presented in Table I and in Figs. 1 and 2. There was a marked increase of thyroidal I^{131} on day 19 of gestation as compared with day 18, and a still further increase on day 20. However, when the radioactivity in the glands was plotted on semi-log paper against time, a straight line was obtained, indicating that the concentration of thyroidal I^{131} increased exponentially each day from 17 to 20. A similar exponential increase in rat thyroidal radioactivity was reported by Vidovic (4), although the initial uptake of I^{131} occurred on day 19 of gestation. A log-log plot of thyroidal radioactivity against body weight and fetal radioactivity also yielded straight-line relationships (Fig. 2). These results demonstrated, therefore, that the concentration of I^{131} by the thyroid was related to an increase of body weight (and presumably thyroid weight) (5, 6), to radioactivity in the fetus, and to gestational time.

Parenthetically it might be noted that the thyroidal concentration of I^{131} in relation to the isotope in the whole fetus was considerably greater than that observed in adult rats. In several species other than the rat, the fetal blood level of I^{131} was greater than that of maternal blood (7–9). Logothetopoulos and Scott (9), however, have stated

briefly that in rats the placenta did not concentrate I^{131}, nor did the fetal blood contain a higher level of I^{131} iodide than did dam blood.

By light microscopy, the morphology of the maturing fetal rat thyroid has been frequently and adequately described (5, 10–12). Suffice it to note that on day 17 of gestation, a few clusters of cells were beginning to be arranged in circular or oval patterns around a minute lumen or cleft. In very few of these lumens a small amount of PAS-positive material was lightly stained. With each additional day of gestation after 17, more and more follicular patterns were found, and more PAS-positive material appeared in lumens, so that by day 20 the fetal thyroid exhibited most of the histologic features of an adult gland (Figs. 3 and 5).

When viewed by fluorescence microscopy, specific staining with the Fl-anti-Tg was observed from day 17 of gestation onward. At this early time, the staining was sparse, appearing as poorly defined minute deposits between cells. On day 18, more stained material was seen, apparently lodged between epithelial cells where they formed a lumen. Occasionally the staining pattern was linear, again between cells. Within the cytoplasm of some groups of cells were fine, almost dust-like granules with specific stain. On day 19, many groups of cells contained more of this intracellular material, and they often surrounded small folicular lumens which showed stained deposits (Fig. 4). On day 20, the follicular spaces were markedly dilated and filled with specifically fluorescent colloid. The cytoplasm of the epithelial cells lacked stain (Fig. 6). The fluorescent picture resembled that of the adult thyroid, in which only colloid exhibits specific green fluorescence.

In the electron microscope, the thyroid at 17 days of gestation was visualized as sheets or clusters of cells delimited by vascular spaces and connective tissues (Fig. 7). The follicular cells were "watery" in texture, had relatively large nuclei, and were apparently quite fragile, since it was extremely difficult to fix and embed them with complete integrity of membranes. Within the cytoplasm all the organelles found in adult follicular epithelium were already present at this time (Fig. 8). The endoplasmic reticulum was represented by varying numbers of rough surfaced circular, oval, oblate, and irregular profiles ranging around $0.4 \times 0.05\ \mu$, dispersed throughout the cytoplasm without apparent connection to one another. The Golgi element was small and composed of the usual morphologic parts described by

FIGURE 3

Thyroid from 19-day fetus, to be compared with Fig. 4. Most of the epithelial cells are arranged around a small lumen containing PAS-positive material. Arrow points to mitosis. × 900.

FIGURE 4

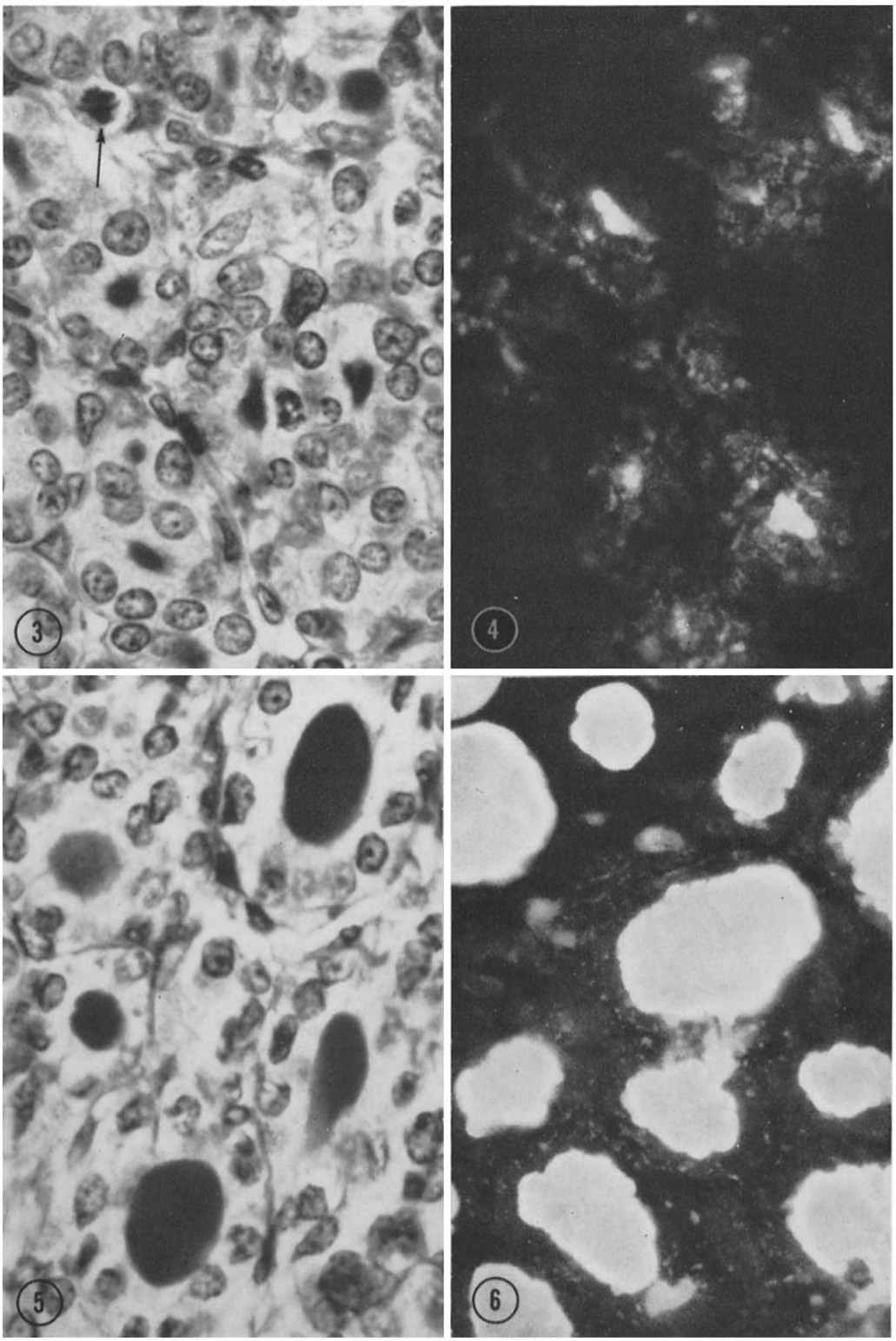
Frozen section of 19-day fetal thyroid stained with Fl-anti-Tg. The large irregular white areas represent specifically stained thyroglobulin in follicular lumens. Note also small scattered granular areas of specific fluorescence in relation to the larger areas representing thyroglobulin deposits in cytoplasm of cells. Fluorescence micrograph. × 900.

FIGURE 5

Thyroid from 20-day fetus, to be compared with Fig. 6. The follicles are well delineated and filled with PAS-positive colloid. PAS stain. × 900.

FIGURE 6

Frozen section of 20-day fetal thyroid stained with Fl-anti-Tg. Large, fairly homogeneous areas of specific fluorescence are seen, indicating thyroglobulin in colloid filling the follicular lumens. Note by comparison with Fig. 4 the lack of specifically stained material in the cytoplasm of the lining follicular cells. Scattered fluorescent dots represent non-specific fluorescence, some of which is in stroma. × 900.


3
4
5
6

others (13). There was no evidence of vacuole formation. Usually a swarm of small vesicles about 400 to 500 A in diameter marked the extent of the Golgi zone. The mitochondria exhibited their characteristics features and were not otherwise unusual. The cell edges were, for the most part, straight and closely apposed to the neighboring cell membrane. There were loci, however, along the cell membrane in which finger-like processes interdigitated with the adjacent cell membrane. Follicular lumens were observed at this time, usually very few and very small, so that they would be difficult to visualize in the light microscope. A few villi projected into them.

As gestation time increased beyond day 17, the epithelial cells exhibited morphologic evidence of maturation, *i.e.*, the nucleus became smaller in relation to total cytoplasm and shifted away from the lumen; the mitochondria enlarged; the Golgi element expanded but showed no evidence of vacuole or droplet formation; and the endoplasmic reticulum began to present the more complex configuration of adult thyroidal structure (Figs. 9, 10, 11, 12, and 15). The follicular lumen was enlarged and was delineated by numerous villi (Figs. 9, 10, and 12). Often, swarms of vesicles slightly larger than those of the Golgi element accumulated in the subvillous area of the cell (Figs. 10 and 13), and occasionally one or two of these vesicles were present in individual villi. Up to day 20 of gestation, droplets were rarely seen in the cytoplasm. When present, they appeared to be lipid in nature and in no way resembled the droplets found in adult thyroid cytoplasm. In day 20 fetal thyroids, however, droplets were found in some epithelial cells (Fig. 14). They varied somewhat in size, shape, and density of contents and were enveloped in a single membrane or lacked such a covering. At this time of gestation, the fine structure of the follicular cell was quite similar to that of adult thyroidal epithelium (14).

Several additional morphologic observations were noted. Terminal bars were seen on day 17 of gestation between some follicular cells and were ubiquitous on day 18. From day 17 to 18, the basement membrane was represented by a thin dense line of the same dimensions as the cell membrane. In some micrographs, a vessel and contiguous follicular cells shared the same dense line between them. By day 19 of gestation, the basement membrane was thicker and composed of an amorphous, moderately dense material similar to basement membrane of adult thyroid.

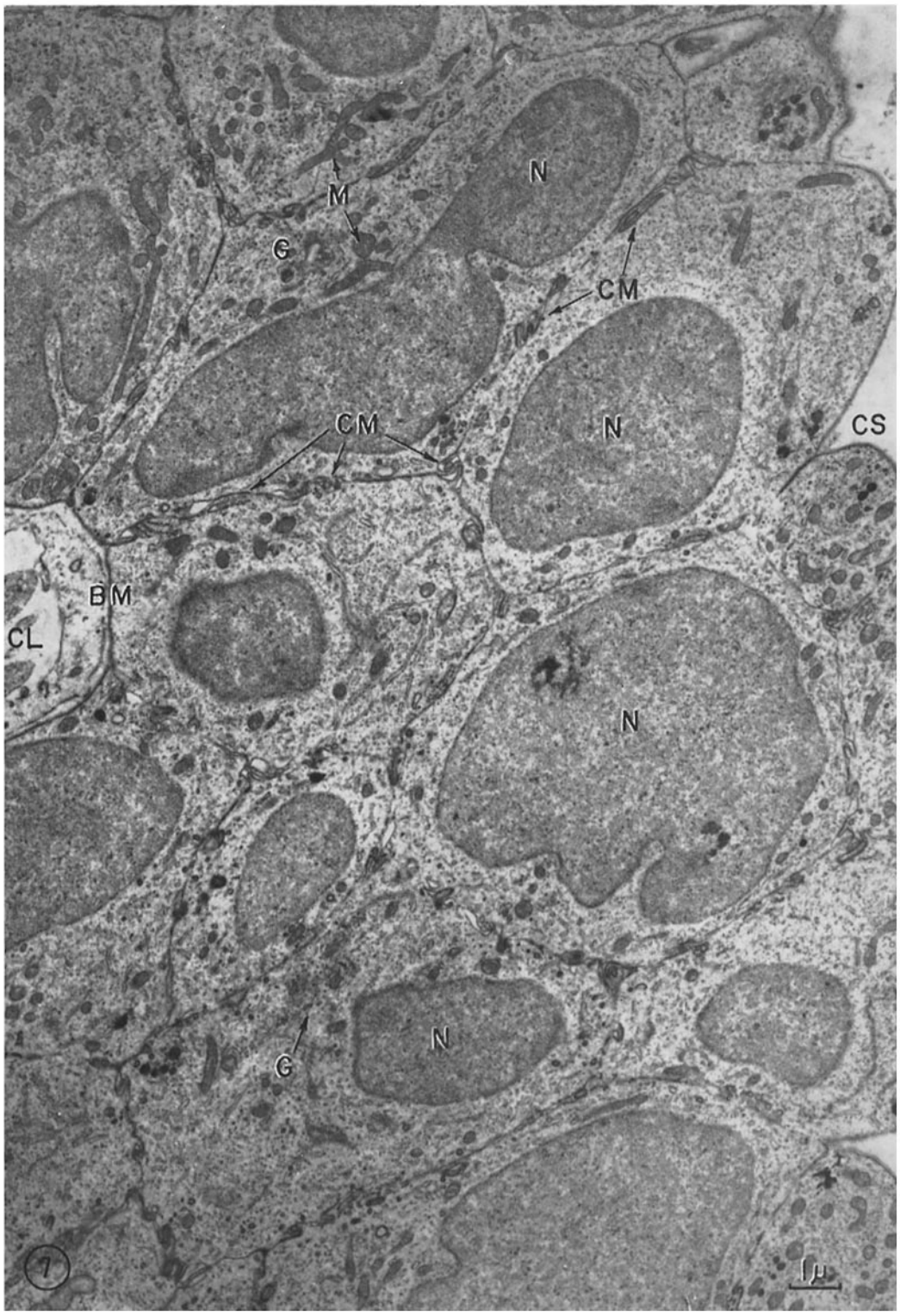
DISCUSSION

When the absolute thyroidal counts per minute were compared for days 18 and 19 of gestation, the difference was striking. There were, however, a small number of fetal thyroids which took up I^{131} on day 18, and an even smaller number which acquired radioactivity on day 17. The thyroidal counts per minute on day 17 were sufficiently low so that one could not be certain of the complete reliability of 40 counts over a background of 250. These low thyroidal counts might not truly represent the concentrating capabilities of the thyroid epithelium, because at day 17, as well as day 18, little of the isotope injected into the dam reached the fetus, and therefore little had the opportunity to arrive at the thyroid. In short, the data of Table I and Fig. 1 and 2 would indicate that the specific function of the thyroid gland, as measured by I^{131} uptake, did not suddenly and precipitously develop within 24 hours but had been present in some glands for one or more days prior to day 19. The exponential development described here for the rat has also been reported previously by Hall and Kaan (5), and for human, bovine, and sheep fetal thyroids by others (15–17).

The ultrastructural findings agreed with the exponential functional development of the thyroid. The follicular cell appeared fully equipped on day

FIGURE 7

Electron micrograph of 17-day fetal thyroid. The cells are arranged in a solid sheet and flanked at one side by a vessel (*CL*) and on the other by connective tissue space (*CS*). A thin basement membrane (*BM*) lies between cells and vessels. The cell membranes (*CM*) outline the cells and interdigitate with adjacent borders (*CM* arrows). The Golgi element is visible at *G*. Mitochondria (*M*) are numerous. The endoplasmic reticulum is not apparent at this magnification. *N*, nucleus. × 8800.


N
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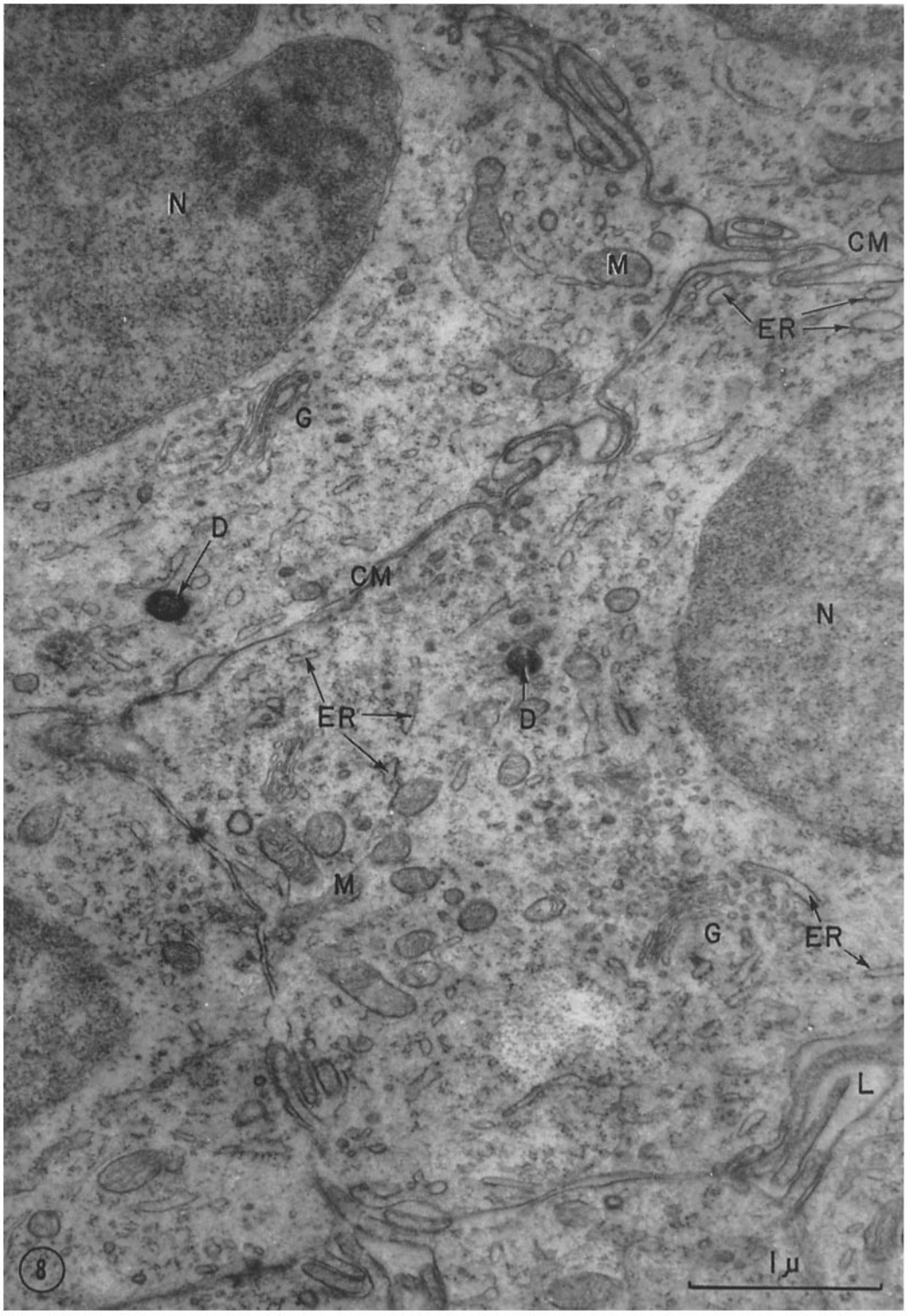
17 in most fetal thyroids before concentration of I^{131} could be measured. It contained mitochondria, Golgi element, and ergastoplasm. As the epithelium matured, none of these organelles became prominent or exhibited morphologic evidence of enhanced activity. During this period, these organelles enlarged *pari passu* with cell growth and became more complex, especially the endoplasmic reticulum, which assumed the configuration of that seen in adult thyroid cells. One could conclude that the cell with all its parts was maturing as a unit both before and after I^{131} concentration could be measured.

By fluorescence microscopy, thyroglobulin or a precursor with similar immunohistochemical specificity was first observed in the thyroids of 17-day fetuses. At this time, the material was sparse and was evident only among a few groups of cells in an extracellular site, most likely in the incipient follicular lumens. Over the next 2 days, the stained material increased in amount. During this period also (days 18 and 19 of gestation), intracytoplasmic stained granules were observed and increased in number and intensity. On day 20, however, intracellular material was not seen. Only the colloid in the follicular lumens stained brightly. This sequence of events suggested that the fetal thyroid cells synthesized thyroglobulin or an immunologically similar protein and retained it in sufficiently high concentration so that it could be visualized within the cell. On day 20, the intracellular thyroglobulin was discharged into the now enlarged lumens and none was observed within the cell. A similar distribution of specific stain is present in the adult rat thyroid. Biologic assay (5) and radiochromatographic studies (18) have shown that rat fetal thyroid does synthesize thyroglobulin, and further, that thyroxine is liberated into the fetal circulation (19). These latter studies were performed on fetal rats beyond 19 days of gestation, and because of the relatively small quantity of I^{131} taken up by the fetal gland before this time, it would be difficult to demonstrate radiochemically the presence of labeled thyroglobulin in the gland or labeled thyroxine in the circulation of day 17 or 18 fetuses. Hall and Kaan (5), however, found thyroxine in day 18 rat fetal thyroids, which would be day 19 according to our determination of gestational time. The loss of intracytoplasmic specific stain and the enlargement of the follicular lumens on day 20 might have been the result of the action of fetal TSH (thyroid stimulating hormone). There have been reports that fetal TSH at this time is discharged from the pituitary and that hypophyseal TSH is efficacious before birth (19–21).

A precise correlation of ultrastructural morphology and intracellular fluorescent granules was not achieved in these experiments. There were no structures in the cell which closely corresponded in size, shape, and distribution to the stained granules seen in day 18 and 19 fluorescent sections. However, it is possible that the stained material was dispersed homogeneously throughout the cytoplasm and that the granular appearance observed in the immunohistochemical preparations resulted from precipitation of this material during the staining procedures. In any event, the concentration of Tg or an immunologically similar reactive compound was sufficiently high so that this protein was visualized within the cell on days 18 and 19 of gestation. After 20 days of gestation, the concentration of Tg within the cell was probably too low for localization with the fluorescence microscope, although the protein was most likely being synthesized intracellularly. The presence of a complex rough surfaced endoplasmic reticulum and its contents indicated that the organelle was the synthetic machinery, particularly in the light of our current knowledge concerning the function of this cytoplasmic structure. The appearance of ultrastructural cytoplasmic droplets on day 20, similar to those found in adult thyroids, was certainly not related to the presence of thyroglobulin

FIGURE 8

Electron micrograph of another 17-day fetal thyroid at higher magnification. Portions of four cells are bounded by cell membranes (*CM*) which interdigitate with neighboring cell borders. An incipient lumen appears at *L*. The Golgi element (*G*) and mitochondria (*M*) are similar to those of adult thyroid cells. The endoplasmic reticulum (*ER*) is represented by a few rough surfaced profiles of various sizes, distributed without pattern in the cytoplasm. Two dense droplets are shown at *D*. × 30,000.


N
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D
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N
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D
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M
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8
1 μ

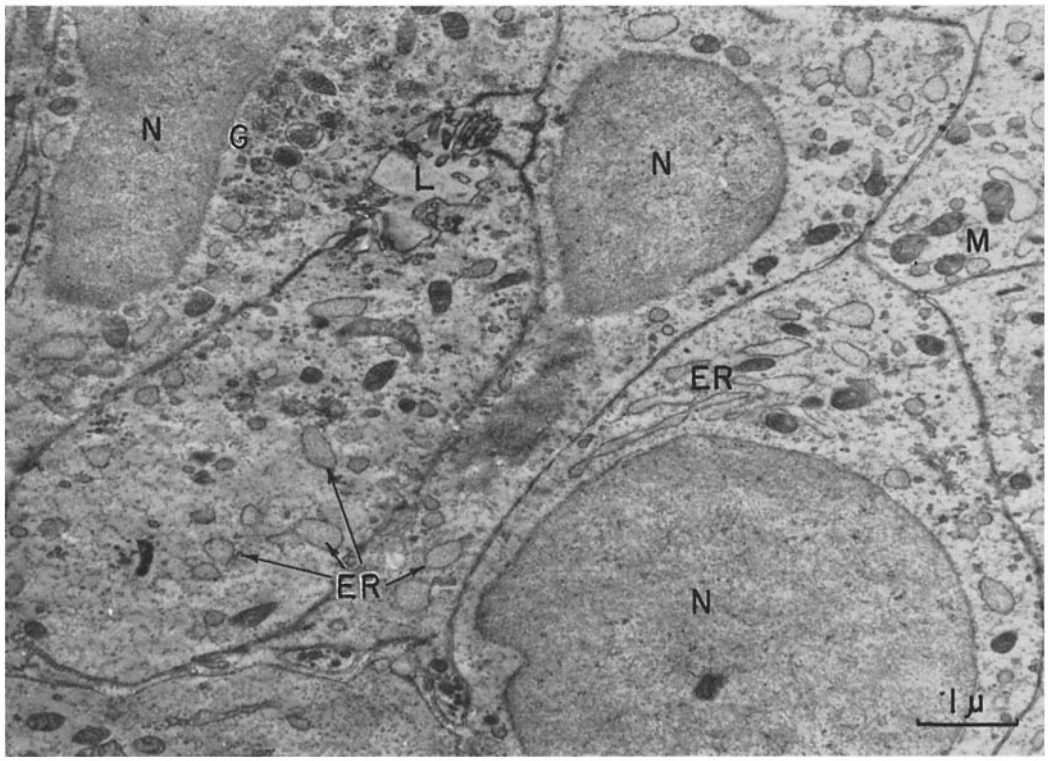

FIGURE 9

Electron micrograph of 18-day fetal thyroid. A small lumen, much smaller than the diameter of a nucleus, is present at *L*. The endoplasmic reticulum (*ER*) is more extensive and elaborate than that seen in Fig. 8. *G*, Golgi element; *N*, nucleus; *M*, mitochondria. × 13,500.

within cells or follicular lumens. The precise chemical composition of these droplets remains unknown, although Stoll and his colleagues believe that in the chick they contain mucopolysaccharides (22).

Most of the 17-day thyroids did not contain I^{131}, and yet these same glands yielded a specific fluorescent reaction indicative of Tg or immunologically similar material. There was in these experiments, however, no way of comparing quantitatively the sensitivity of the isotope technique with the sensitivity of the fluorescence technique. Although our observations suggested that Tg or a precursor was synthesized in the absence of iodide within the cell, no definitive conclusion could be reached on this matter. Nadler *et al.* believe that "typical," *i.e.* iodinated, Tg is formed in the follicular lumen (23). The present investigation also did not determine the locus of iodide binding, *i.e.* cellular or luminal, about which there is not yet unanimity of opinion (24–26).

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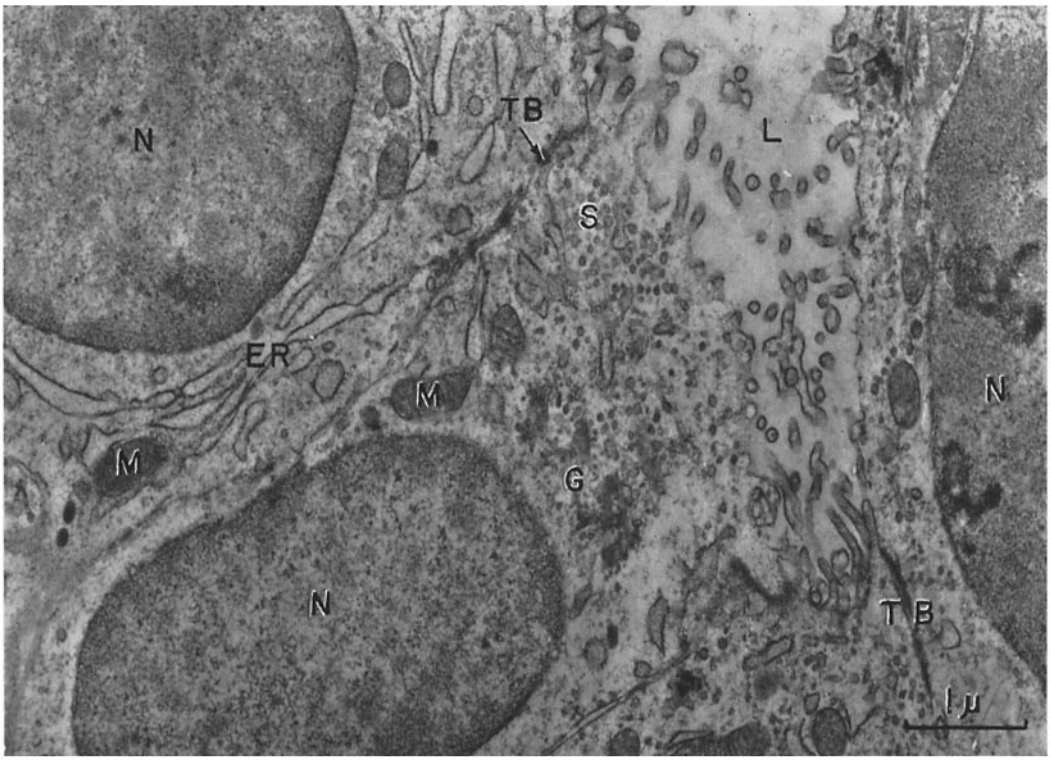

FIGURE 10

Electron micrograph of another 18-day fetal thyroid. The lumen (*L*) in this photograph is much larger than the one shown in Fig. 9. Villi project into it. A terminal bar (*TB*) marks the apposition of two cells at the lumen. A swarm (*S*) of vesicles occupies a zone of cytoplasm just beneath the villi. The Golgi element (*G*), mitochondria (*M*), and endoplasmic reticulum (*ER*) are more mature than the corresponding organelles of the day 17 thyroid. *N*, nucleus. × 20,000.

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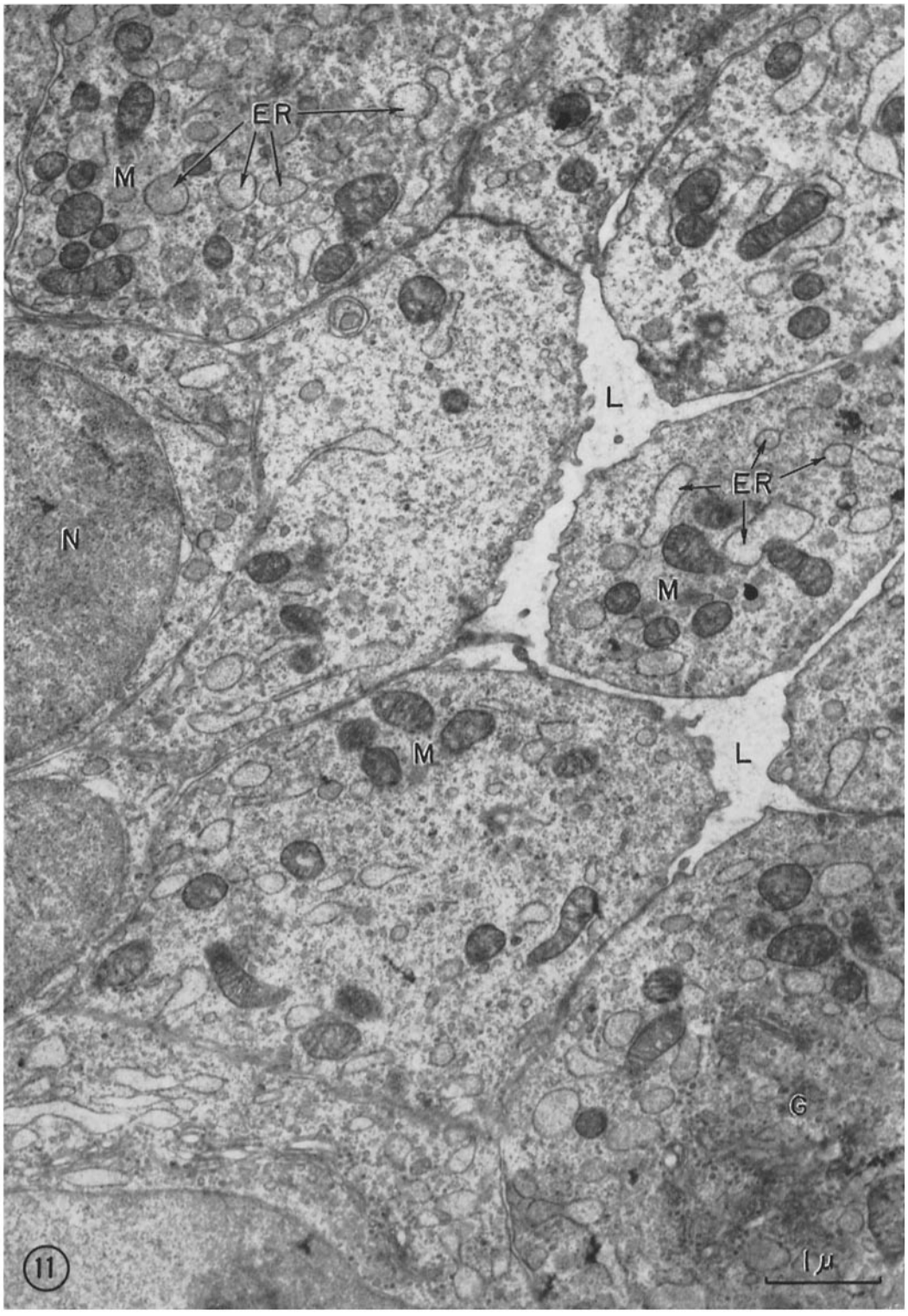
FIGURE 11

Electron micrograph of 18-day fetal thyroid. An irregular cleft (*L*) between cells marks the site of a follicular lumen. Within is a light concentration of granular material. The endoplasmic reticulum (*ER*) is composed of dilated rough surfaced profiles which contain a granular precipitate. Within the cytoplasm are numerous minute smooth surfaced vesicles and dispersed ribonucleoprotein particles. Abbreviations as in preceding figures. × 20,000.


ER
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L
ER
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L
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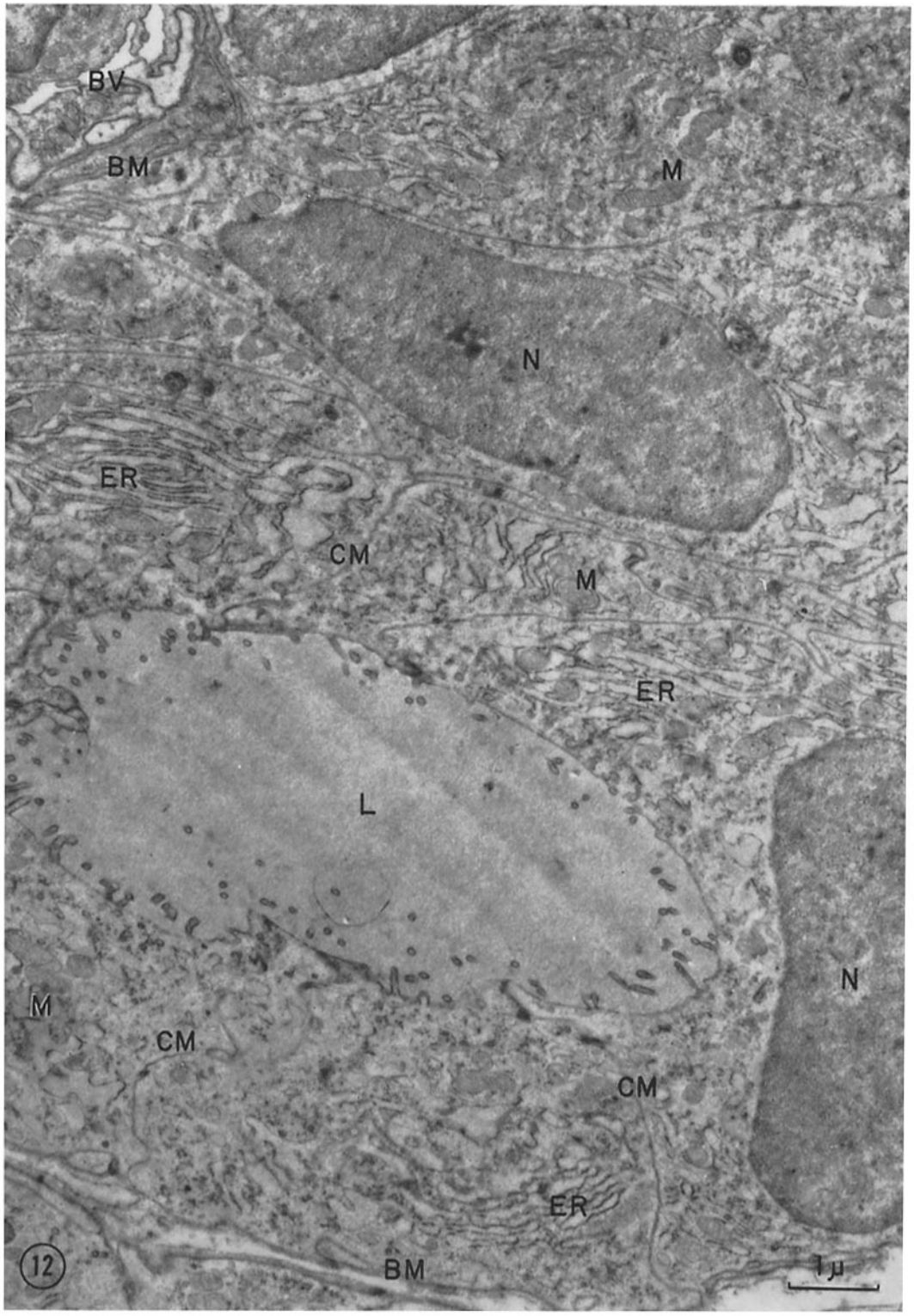
11
1 μ

FIGURE 12

Electron micrograph of 19-day fetal thyroid. Numerous villi project into the lumen (*L*), which contains a granular precipitate. In the surrounding cells a well developed rough surfaced endoplasmic reticulum is seen. A blood vessel (*BV*) is separated from epithelium by a basement membrane (*BM*). Abbreviations as in preceding figures. × 14,000.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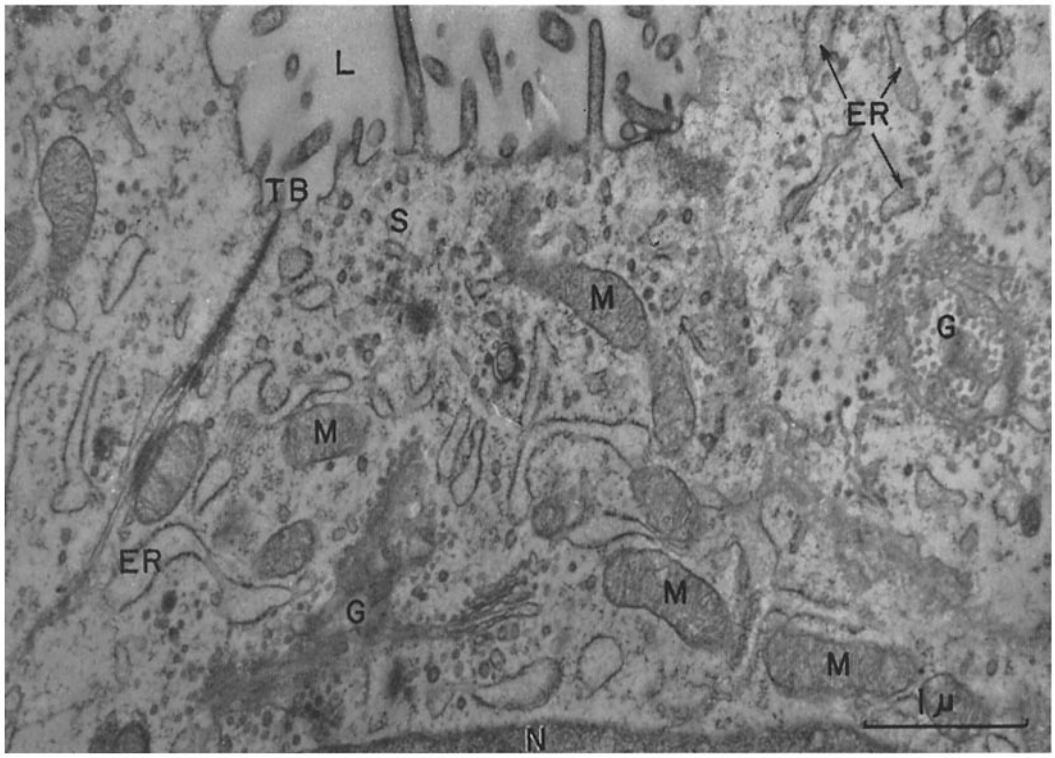


FIGURE 13

Electron micrograph of 19-day fetal thyroid. The morphology at this time is quite similar to that seen on day 18. The lumen (*L*) is larger. In the subvillous zone is a swarm of vesicles (*S*) slightly larger than similar vesicles in the Golgi element (*G*). The endoplasmic reticulum (*ER*) begins to resemble that of the adult, and within its sacs is a granular precipitate. Abbreviations as in preceding figures. × 20,000.

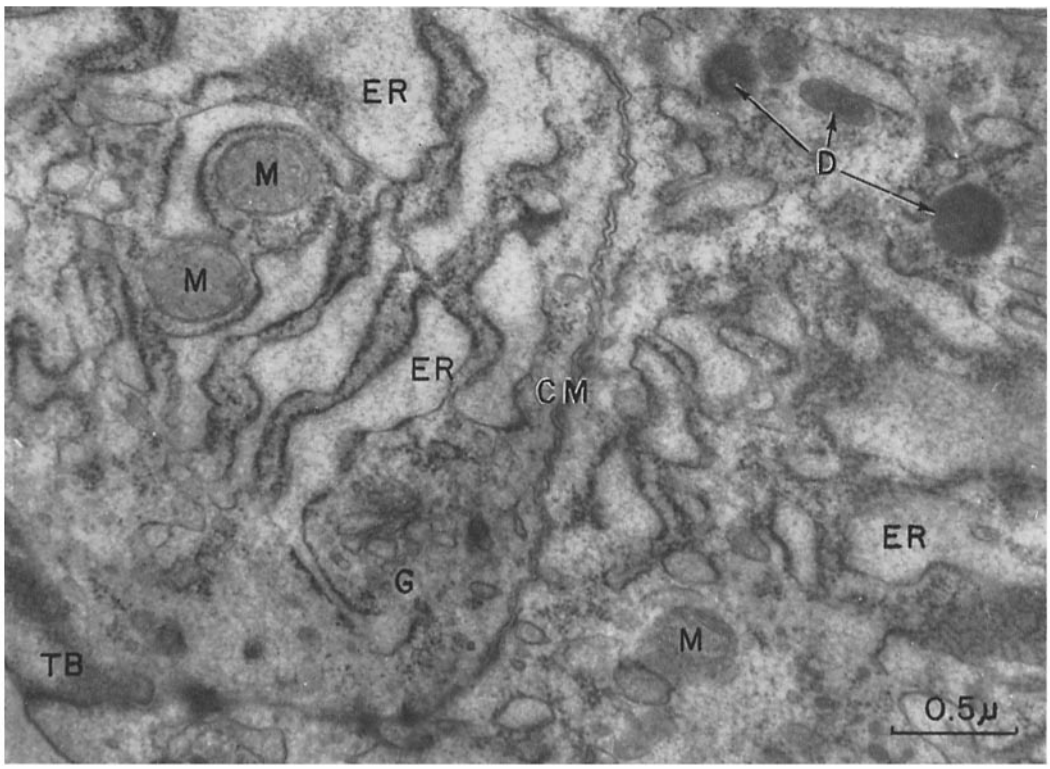
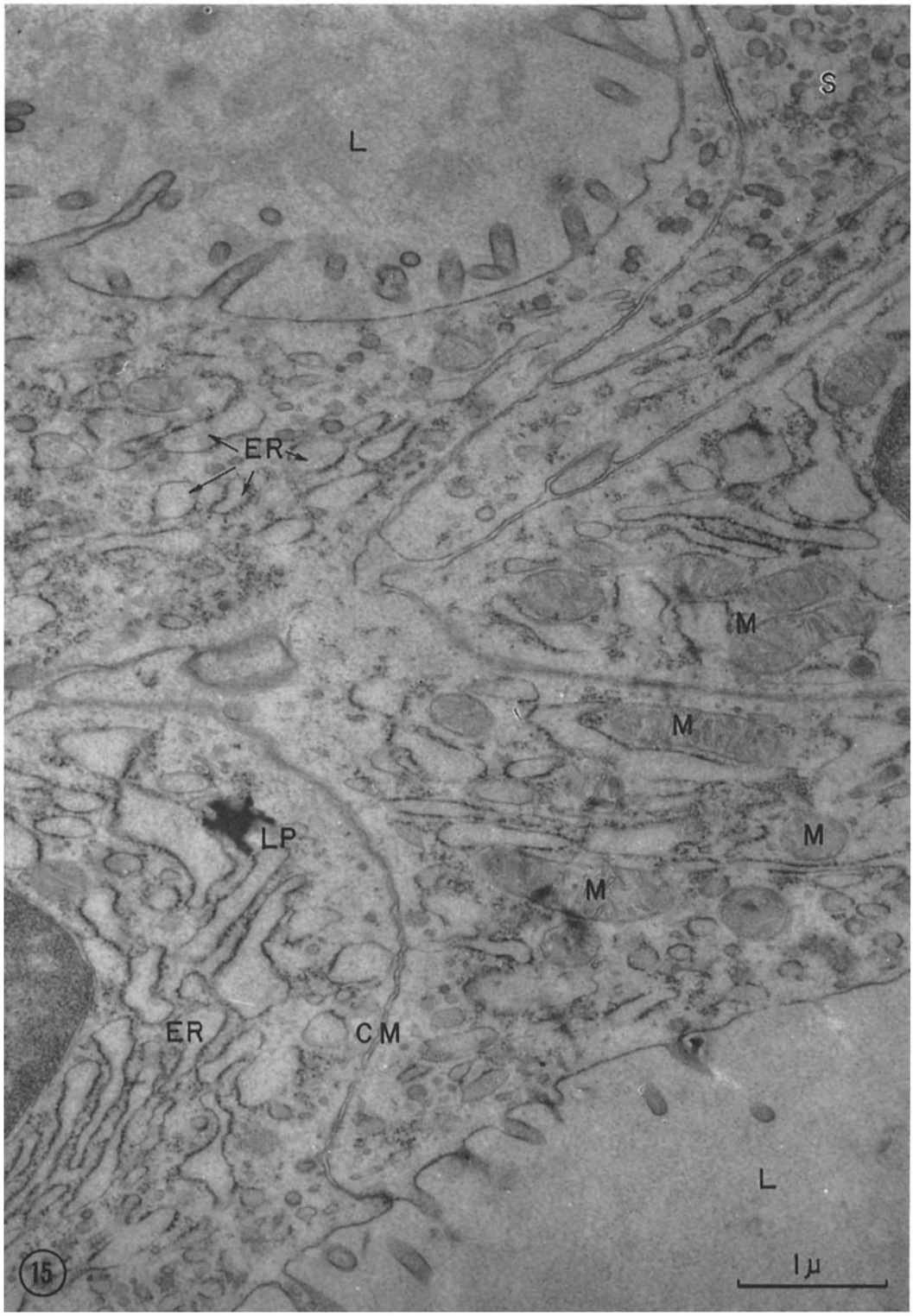


FIGURE 14

Electron micrograph of a portion of thyroid from a 20-day fetus at higher magnification. Three droplets are shown at *D*, and the central one is lined by a single membrane. The other organelles and cell structures are marked as in preceding figures. × 34,000.

FIGURE 15

Electron micrograph of thyroid from 20-day fetus. The lumens (*L*) are now quite large in comparison with cell size (see Figs. 9 and 11). The endoplasmic reticulum (*ER*) at this time is composed of elongate, occasionally dilated, connected sacs. Its contents are much less dense than those of the follicular lumens. A cluster of smooth surfaced vesicles is located near the lumen at *S*. A lipid particle is seen at *LP*. Except for the absence of droplets, the morphology is similar to that of adult thyroid epithelium. Abbreviations as in preceding figures. × 22,500.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