established between cell positions 6–7 and $G_2 + M$ takes 1.5 h, then labeled daughter cells migrated from their point of origin to the villus base at a rate of 1.3–1.5 cell positions per h.

Discussion. The distribution of cells in DNA synthesis immediately after 3HTdr injection (Figure 4) suggests that the cryptal population can be subdivided into a region of high proliferation (cell positions 8–14) and a region of cell specialization (cells 1–8). Similar observations have been reported by other investigators[7–9]. However, if attention is focused on the progenitor cells positioned in the deeper regions of the crypt, it appears that an asymmetrical division does not necessarily account for the maintenance of the steady state in the mouse duodenum.

If the rate of cell migration (1.3–1.5 cell positions per h) presented in Figure 3 is compared to the distribution of proliferating cells (Figure 4), it appears that a symmetrical division may be the most common form in the deeper portions of the mouse cryptal epithelium. For example, daughter cells that are produced deep in the crypt (cell positions 10 through 14) can only migrate from 5.2–6.0 cell positions during the G_1 (duration of 4 h) following the division that produced them. Therefore, these progeny arrive in the region (cell positions 5 through 9) conducible to a high probability of proliferation at a time when they are about to enter a new S-phase[7,8,10]. In addition, if the progenitor cells present in the region below cell 14 are considered, e.g., the Paneth zone[12], progeny of these divisions could remain in the crypt for 2 or more cell cycles before they are able to migrate into the regions of cell specialization. Thus, it is concluded that divisions of progenitor cells deep in the crypt will probably produce 2 progenitor cells that will at least enter one more division cycle before they specialize. In order to compensate for this, an equal number of divisions that produce 2 specialized progeny must occur. Inspection of Figures 3 and 4 reveals that such a division could possibly occur beginning with cell positions 9 or 10. Thus, the cutoff point for the decision between 2 types of daughter cell production must lie between cell positions 9 and 10. This is reasoned because the cells most likely to specialize immediately after division and during the short G_1 phase (4 h) are those present in the upper regions (cell positions 1–10). Furthermore, it should be emphasized that a progenitor division could also give rise to 2 progeny, one specialized and the other a progenitor. This phenomenon would depend upon the position of the dividing cell, the rate of migration, the intensity of a possible feedback inhibition, and the final position of the daughter cells before DNA synthesis is either initiated or precluded[7,10].

It has been suggested but not conclusively demonstrated that all cryptal cells are potentially progenitor cells[7,10,11]. Thus, analysis of the changes in the proliferative vs. the maturation zones under a variety of experimental conditions may lead to a greater understanding of those mechanisms that bring about cell specialization[9].

Zusammenfassung. Der Zusammenhang zwischen der Teilung von Ursprungszellen und der Differenzierung ihrer Tochterzellen wurde anhand von Epithelzellen des Duodenums weisser Mäuser untersucht. Um den Zustand des «steady state» zu erhalten, finden die folgenden Arten von Teilung der Ursprungszellen statt: 1) zwei neue Ursprungszellen; 2) zwei differenzierte Tochterzellen; oder 3) eine neue Ursprungszelle und eine differenzierte Tochterzelle.

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Experimental Goiter: Ultrastructure and Autoradiography

Goiters can be induced experimentally by the prolonged feeding of a deficient-iodine diet in many laboratory animals. Previously, ultrastructure and secretory cycle of thyroid cells in control animals have been extensively studied (EKHOLM[1], WISSIG[2], LUPULESCU and PETROVICI[3]), but little is known about the fine structure of goiters (FELDMAN[4]). In particular, few have attempted a correlation between the ultrastructure, radioautography and radiochromatograms in goiter induced by low iodine diet. In spite of the recent interest in other goitrogenic factors, chronic iodine deficiency remains the main cause of goiter.

Biochemical disorders revealed that the triiodothyronine/thyroxine (T3/T4) ratio increases progressively in the follicular cells of the iodine-deficient rat thyroid (STUDER and GREER[5]).

This study deals with the ultrastructural pattern of the iodine-deficient goiters in rats and with the intracellular distribution of radioiodinated proteins.

Materials and methods. For induction of goiter male Wistar rats, weighing 180–200 g, were used. The first group, the controls, received a stock laboratory diet, containing 0.9–1.2 μg I/g food. The second group was fed for 4 months with a modified-Remington low iodine diet, containing 0.03–0.04 μg I/g food.

Radioiodine (^{125}I) was injected i.p. (275 μC) into 5 rats, 2–6 h before sacrificing, for light and electron microscopic autoradiography. The other 15 from each group received i.p. injections of 12 μc ^{131}I for chromatographic analysis of thyroid tissue. For ultrastructural pattern of the

[1] R. EKHOLM, in *Electron Microscopic Anatomy* (Ed. S. KURTZ; Academic Press, New York 1964), p. 221.
[2] S. WISSIG, in *The Thyroid Gland* (Eds. R. PITT-RIVERS and W. TROTTER; Butterworths, London 1964), vol. 1, p. 32.
[3] A. LUPULESCU and A. PETROVICI, *Ultrastructure of the Thyroid Gland* (The Williams and Wilkins Co., Baltimore 1968).
[4] J. FELDMAN, in *Advances in Thyroid Research* (Ed. R. PITT-RIVERS; Pergamon Press, Oxford 1961), p. 318.
[5] H. STUDER and M. GREER, *The Regulation of Thyroid Function in Iodine Deficiency* (Hans Huber, Bern and Stuttgart 1968), p. 127.

thyroid, the specimens were fixed in veronol-buffered osmium tetroxide and then embedded in Vestopal-W or Epon-812. The grids were stained with lead citrate and examined under electron microscope. For electron microscopic radioautography, CARO's and VAN TUBERGEN's[6] method was used.

The biosynthesis of hormones were studied using TONG and CHAIKOFF[7] procedure. Pronase was used as the hydrolitic enzyme and iodine containing compounds were separated by ascending chromatography.

Results. Electron microscopic observations showed typical secretory cells in control rat thyroid (Figure 1). In iodine-deficient goiters, there are interesting ultrastructural changes, including hypertrophic microvilli, small apical vesicles (Figure 2) and many dense granules or oval bodies. The rough endoplasmic reticulum (RER) was distended and there were many confluent ergastoplasmic sacs or cisternae. This distension causes a severe disorganization of the ultrastructural organelles (Figure 3), which consisted of swollen mitochondria, large colloid droplets, and many free polysomes arranged in 'rosettes'. Other changes involved enlarged nuclei rich in chromatin and the Golgi complex was hypertrophied.

Electron microscopic radioautographs demonstrated that most of the developed grains of ^{125}I were found over dense droplet, endoplasmic reticulum and apical cell border (Figure 4).

Radioiodine uptake (^{131}I) showed a marked increase in thyroid uptake in the goitrous rats which accumulated 75% of administered dose as compared with the controls only 25%. Goitrous rats have a significant increase of labeled monoiodotryrosine (MIT) and triiodothyronine (T3); but a decrease of diiodotyrosine (DIT) and thyroxine (T4) (Figure 5).

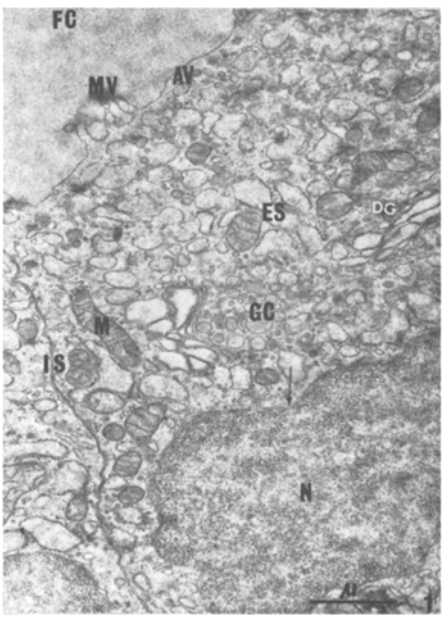

Fig. 1. Control rat thyroid. 2 follicular cells, separated by intercellular space (IS); FC, follicular colloid; MV, microvilli; AV, apical vesicles; ES, ergastoplasmic sac; M, mitochrondrion; GC, Golgi complex; N, nucleus with pores (arrow); DG, dense granule. × 22,000.

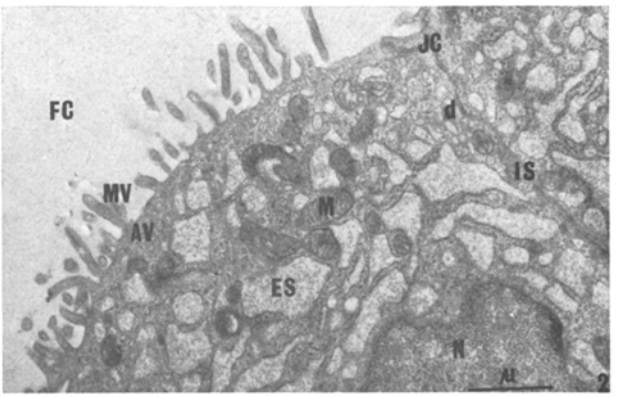

Fig. 2. Goiter due to low iodine diet-hyperplastic and hypertrophied microvilli (MV); many apical vesicles, colloid is reduced; d, desmosome JC, junctional complex; other abbreviations as in Figure 1. × 22,000.

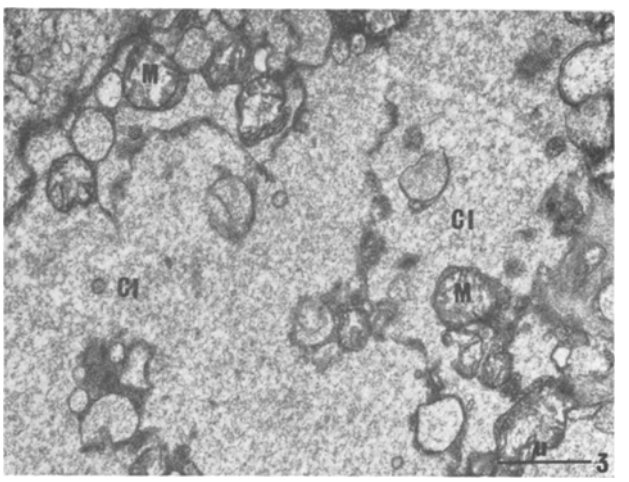

Fig. 3. Goiter due to low iodine diet. There is great distension and disorganization of the endoplasmic reticulum (middle zone of thyroid cell); several cisternae (CI) containing in their lumen a condensed material, have become confluent, swollen mitochrondria (M); other abbreviations as in Figure 1. × 22,000.

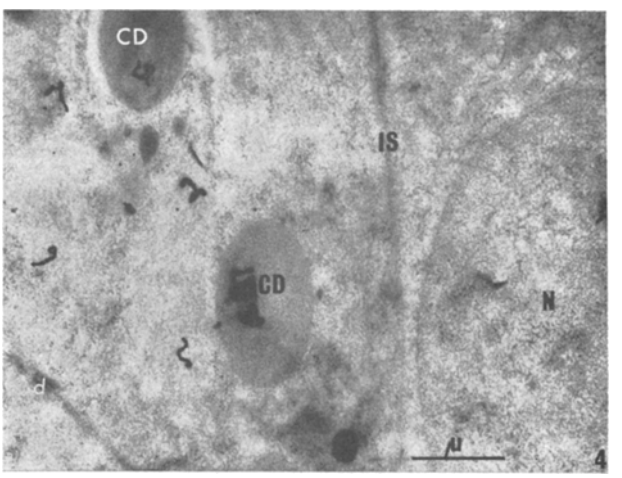

Fig. 4. Goiter due to low iodine diet. Electron microscopic radioautograph 6 h after ^{125}I. Developed grains are located over the dense droplets (CD) and also over the ergastoplasmic sacs. × 22,000.

[6] L. CARO and R. VAN TUBERGEN, J. Cell Biol. *15*, 173 (1962).
[7] W. TONG and T. CHAIKOFF, J. biol. Chem. *232*, 939 (1958).

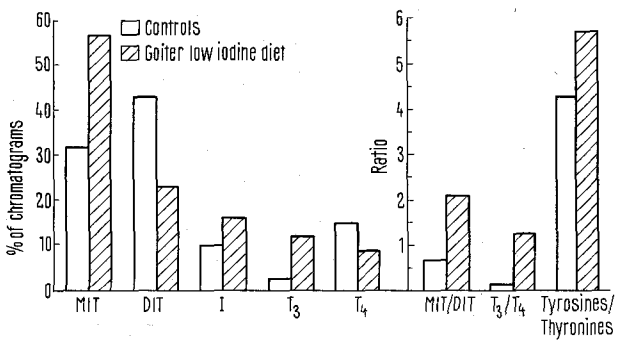

Fig. 5. Chromatographic distribution of labeled iodoaminoacids in dense rat and in goiter by low iodine diet. MIT, monoiodotyrosine; DIT, diiodotyrosine; T3, Triiodotyroxine; T4, thyroxine; MIT/DIT, ratio; T3/T4, ratio.

Discussion and conclusions. In this study, the ultrastructural changes were correlated with radioautography and biochemical disorders for a better understanding of pathogenesis of goiter. The most significant changes were extensive enlargement of the endoplasmic reticulum (RER), increased in number of colloid droplets and of dense granules, hypertrophy of microvilli and Golgi apparatus. Radioautography showed a greater avidity of these goiters for radioiodine, the developed grains were located at the peripheral colloid and intracellularly. Radiochromatograms revealed the predominance of MIT over DIT and T3 over T4. The enlargement of RER is probably related to increased protein synthesis but the possibility of a defective transport of thyroglobulin through the endoplasmic channels cannot be ignored. This enlargement of endoplasmic reticulum indicates that the cellular machinery can operate actively in goiter. Another interesting finding is the increased number of dense granules or lysosomes referred to as 'zymogen-like' granules; these contain acid phosphatase and probably are involved in proteolysis of thyroglobulin. The evidence in this report supports the argument that fine structural changes in iodine-deficient thyroids are induced by endogenous TSH-overstimulation, as well as by a lack of iodine.

Thyroglobulin iodination takes place at the cell-colloid periphery, many developed grains may also be scattered over the dense droplets and ergastoplasmic sacs. This intracellularly iodination as well as the predominance of MIT and T3 over DIT and T4 respectively, points to a homostatic cellular mechanism that works to spare the iodine and synthesize less iodinated hormones in goiters.

Résumé. Une étude corrélative concernant les modifications ultrastructurelles, autoradiographiques et biochimiques a été faite dans le goître expérimental provoqué par une carence prolongée en iode chez les rats. Il s'est produit une dilatation et une désorganisation extensive du réticulum endoplasmique, accompagnées d'une augmentation des granules denses, des gouttes colloïdales, de même qu'une hypertrophie de l'appareil de Golgi et des microvillis. L'autoradiographie met en évidence une iodination intracéllulaire.

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Failure to Produce Tumours in Cattle with 20-Methylcholanthrene and 3,4-Benzpyrene

We failed to find in the available literature[1,2] any report about the effects of chemical carcinogens like 20-methylcholanthrene (MCA) and 3,4-benzpyrene in cattle. Tumours induced in mice and rats by these carcinogens are known to possess distinct antigens[3]. In the course of an investigation designed to study the specific antigens of chemically induced tumours, 1 member each from 1 identical twin pair (female, Jersey breed) and 2 fraternal, female–female, twin pairs (one Jersey and one Friesian breed) were injected s.c., behind the left flank, with 400 mg of MCA suspended in olive oil. At the time of this injection, these animals were 4–6 months old; 315 days later, a gelatin capsule containing 250 mg of powdered MCA was implanted into each animal at approximately the same site as the original injection. One member each from 2 other identical twin pairs (female, one Friesian and one Guernsey breed) received under anaesthesia one 5 mg pellet each of 3,4-benzpyrene under the capsule of the right kidney. 10 weeks later, a gelatin capsule containing 250 mg of powdered MCA was implanted behind the left flank of these 2 animals also. These 2 animals were about one year old at the beginning of the experiment.

All the animals were housed in a grassy paddock more than an acre in area. They were fed ad libidum with green grass, Lucien hay and crushed oats, and were regularly provided with salt lick and were subject to regular physical examination. Blood smears from these cattle were examined immediately before their exposure to the carcinogens, then once every 6 months and at the time of killing. The 2 cows which received benzpyrene pellets in their kidney were subjected to i.v. pyelography and needle biopsy of the pellet implanted kidney about 6 months after implantation of the pellets. About 1 month after the biopsy, exploratory laparotomy was performed. After the laparotomy, both these animals developed pulmonary complications; 1 of them died a week later and the other was killed after a month. The other 3 animals and their co-twins were killed 36 months after the beginning of the experiment. Autopsy was performed on all animals and tissues were examined histologically. Apart

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2 J. E. MOULTON, Ann. N.Y. Acad. Sci. *108*, 620 (1963).
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