THE ELABORATION OF PROTEIN AND CARBOHYDRATE
BY RAT PARATHYROID CELLS AS REVEALED
BY ELECTRON MICROSCOPE RADIOAUTOGRAPHY

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ABSTRACT

The parathyroid glands of young rats were radioautographed after a single injection of the protein precursor tyrosine-\(^{3}H\) in the hope of identifying the sites of synthesis and migration of newly formed protein in the gland cells. The same procedure was used after injection of the glycoprotein precursor galactose-\(^{3}H\). As early as 2 min after intravenous injection of tyrosine-\(^{3}H\), the label was mainly found in the rough endoplasmic reticulum suggesting that cisternal ribosomes are sites of protein synthesis. By 5 and 10 min, much of the label had migrated from the rough endoplasmic reticulum into the Golgi apparatus. By 20 and 30 min, some label had migrated from there into secretory granules. By 45 min and 1 hr, the label content of the cell had decreased, indicating release of labeled material outside the cell. At 2 min after intravenous injection of galactose-\(^{3}H\), the label was mainly present in the Golgi apparatus, where presumably galactose is taken up into glycoprotein. By 10 min, some label appeared in secretion granules and by 30 min release of the material to the outside of the cell was under way. In conclusion, it is likely that the tyrosine-labeled protein material consists mainly of the parathyroid hormone. The galactose-labeled carbohydrate material would be either associated with the hormone in the cell or be part of a distinct glycoprotein which may be the one present on the outer surface of the plasma membrane (cell coat).

The parathyroid gland of the rat is composed of a single type of cells (1–5). These are similar to the chief cells of man (6–8) and other species (9–22), and are presumed to elaborate the hormone of the gland. This hormone has been identified as a protein (23); and active protein synthesis in the cells has been suggested by radioautographs showing uptake of label from bicarbonate-\(^{14}C\) (Fig. 16 in Reference 24) and methionine-\(^{35}S\) (25). However, these results were observed in the light microscope, so that the sites of synthesis and migration of protein within the cells could not be identified. The present work is an attempt to trace newly synthesized proteins in parathyroid cells using electron microscope radioautography after injection of tritium-labeled tyrosine, an amino acid component of the parathyroid hormone (26, 27).

The hormone is believed to be composed of a polypeptide chain without carbohydrate side chains (23, 27). However, a light microscope examination of parathyroid cells in the monkey showed fairly large granules which, because of their staining with the periodic acid-Schiff technique, were presumed to contain carbohydrate (13). It was thought at the time that these might be secretion granules and that the parathyroid gland, like many others (28), might secrete a glycoprotein. Galactose-\(^{3}H\) was then injected to find out whether its label was taken up.
Ficini Radioactivity present in the acid-soluble fraction (presumably due to free tyrosine-3H) of plasma (solid line) and liver (dotted line), in rats sacrificed at various times after injection of tyrosine-3H.

into this hypothetical glycoprotein and, if so, to trace its migration by radioautography. However, when parathyroid cells were examined in the electron microscope, it was realized that the secretion granules are too small to be readily resolved in the light microscope and that the periodic acid-Schiff-stained granules are probably lysosomes. Nevertheless, the radioautographs demonstrated a substantial uptake of galactose-3H label and its migration within the organelles of parathyroid cells.

MATERIAL AND METHODS

1 mCi of L-tyrosine-3,5-3H (The Radiochemical Centre, Amersham, Buckinghamshire, England; SA, 28.2 Ci/mmole) was injected into the external jugular vein under ether anesthesia using groups of two animals each, which were sacrificed 2, 5, 10, or 20 min later. The same dose was injected intraperitoneally into groups of two animals each, sacrificed 30, 45, or 60 min after injection, this route being used to avoid anesthetizing them twice, once for the injection and later for sacrifice. The sacrifice was by perfusion of 4% formaldehyde in Sorensen's buffer at pH 7.3, a fixative known to cause only a low level of binding of free amino acid (29, 30). The dissected parathyroid glands were removed, postfixed in 1% buffered osmium tetroxide, and embedded in Epon.

In a parallel experiment run under similar conditions after injection of tyrosine-3H, male albino rats weighing 38 ± 3 g received an intravenous injection of about 4.5 mCi of α-galactose-1-3H (The Radiochemical Centre, SA, 1.17 Ci/mmole). The animals were again sacrificed by perfusion via the left ventricle using either 4% formaldehyde or 2.5% glutaraldehyde, both buffered with sodium phosphate at pH 7.2-7.3, with additional 0.1% sucrose and 1% galactose. This procedure was carried out for two animals each at 2, 10, 20, and 30 min respectively, after injection. The parathyroids were postfixed in cold 1% OsO4 and embedded in Epon. Semithin (0.5 μm) Epon sections were radioautographed (31) using an exposure of 4 wk after tyrosine-3H and 10 days after galactose-3H injection; and silver grains were enumerated per unit area (Tables I and II). Grey-to-silver sections were radioautographed for electron microscopy (32-35), in which case exposure was for 7 months after tyrosine-3H and 2 months after galactose-3H injection. Radioautographs were developed in Elon-ascorbic acid with previous gold latensification for 5-6 min at 24°C. Staining was with ethanolic uranyl acetate for 20 min followed by lead citrate for 35 min.

One hundred electron microscope radioautographs were taken at each time interval in both the tyrosine-3H and galactose-3H experiments and printed at a magnification of 30,000. Counts were obtained on 20 photographs at each time interval by the method of Whur et al. (36). The organelles identified within a "resolution boundary circle" of 6.5 μm radius drawn around each grain were given the point rating of.

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TABLE I
Concentration of Radioactivity in Parathyroid Cells at Various Times after Injection of Tyrosine-3H*

| Time after injection (min) | Grain count/100 µ² (after deduction of background) ± standard error |
|---------------------------|---------------------------------------------------------------|
| 2                         | 7.3 ± 0.2                                                    |
| 5                         | 8.8 ± 0.2                                                    |
| 10                        | 9.9 ± 0.3                                                    |
| 20                        | 6.5 ± 0.2                                                    |
| 30                        | 6.2 ± 0.2                                                    |
| 45                        | 4.4 ± 0.2                                                    |
| 60                        | 4.4 ± 0.2                                                    |

* As measured by grain counts per unit area in light microscope radioautographs of 0.5-µ Epon sections.

TABLE II
Concentration of Radioactivity at Various Times after Injection of Galactose-3H*

| Time after injection (min) | Grain count/100 µ² (after deduction of background) ± standard error |
|---------------------------|---------------------------------------------------------------|
| 2                         | 5.7 ± 0.1                                                    |
| 10                        | 12.2 ± 0.4                                                   |
| 20                        | 11.0 ± 0.4                                                   |
| 30                        | 12.3 ± 0.5                                                   |

* As measured by grain counts per unit area in light microscope radioautographs of 0.5-µ Epon sections.

Whur et al. (36) (i.e., one, one-half, or one-third, according to whether there were one, two, or three organelles within the circle). The results are found in Fig. 10 for tyrosine-3H and Fig. 13 for galactose-3H.

These methods showed reaction not only over rough endoplasmic reticulum (rER), Golgi apparatus, and secretion granules, but also over plasma membrane and mitochondria. It was possible that some of these reactions were due to the incorporation of tyrosine label within diffusely distributed structures, such as free ribosomes or ground cytoplasm, rather than within the organelles under study; there was also the chance of a small amount of binding of free labeled precursor; finally, there was a very low background fog. All these, it was assumed, would occur at random. The method devised by Whur et al. (36) to minimize the effects of any "random" grain distribution and thus determine whether each organelle takes up a significant amount of label was applied to the present problem. After the organelles associated with each silver grain had been identified within the resolution boundary circles, the same photographs were used to place circles of 6.5 mm radius at random, and the organelles included in these random circles were again rated as done by Whur et al. (36) (Table III A). The percentage obtained for each organelle in this operation was subtracted from the percentage of silver grains over that organelle. The results are given in Tables III B and III C. Finally, the relative concen-
FIGURE 2  Light micrograph of 0.5 μ Epox section from formaldehyde-fixed parathyroid gland of rat. Toluidine blue staining. The parenchyma of the parathyroid gland is composed of compact clusters and cords of polyhedral or slightly elongated chief cells. Between the cords is an irregular network of loose connective tissues containing capillaries. X 280.

FIGURE 3  Light microscope radioautograph of the rat parathyroid gland 2 min after injection of tyrosine-3H. Toluidine blue poststaining. Numerous silver grains are distributed homogeneously over the parenchymal cells. Few grains are present over the connective tissue. X 670.

The concentration of radioactivity was measured in the tyrosine-3H experiment by dividing at each time interval the grain count over a given organelle by the volume of this organelle as measured by Chalkley's method (37) at that interval. The results are presented in the Discussion as Fig. 14.

RESULTS
The rat parathyroid gland is composed of clusters and cords of parenchymal cells (Fig. 2) lined by a basement membrane which separates them from bands of connective tissue containing capillaries.

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Parathyroid cells are small, polyhedral, and compactly arranged (Fig. 4). They contain scattered cisternae of rough endoplasmic reticulum (RER) and numerous free ribosomes, rather small mitochondria, and, occasionally, microtubules, multivesicular and dense bodies (Fig. 8, L). The Golgi apparatus is composed of several stacks made up of a few, somewhat distended saccules; their concavity contains circular and tubular profiles, coated vesicles, and secretion granules (Fig. 5). Secretion granules (1500–2500 Å) are also present in the neighborhood in variable numbers and may be associated with coated vesicles as well as with the plasma membrane (Fig. 5).

**Tyrosine-3H Experiment**

The acid-soluble radioactivity in blood plasma and liver (Fig. 1) showed a rapid drop between 2 and 10 min after injection of tyrosine-3H. Presumably then, the pulse of precursor available to the tissues was brief.

In light microscope radioautographs, the silver grains were fairly homogeneously distributed at all time intervals (Fig. 3). The number of silver grains increased slightly up to 10 min and decreased to 44% of the peak by 60 min after tyrosine-3H injection (Table I).

In electron microscope radioautographs obtained at the earliest interval (2 min) most of the silver grains were over profiles of RER (Figs. 6 and 7). A few silver grains occurred over Golgi apparatus and plasma membrane. Very few were over mitochondria, secretion granules, and nucleus.

At 5 min after injection, silver grains were again seen over the rER but their frequency seemed to decrease; and many grains appeared over the stacks of saccules of the Golgi apparatus (Fig. 8). By 10 min there seemed to be further decrease over the rER and increase over Golgi saccules.

At the 20- and 30-min intervals, the number of silver grains dropped to a low level over the rER, while a significant number was present over the Golgi apparatus either over the saccules or the concavity of Golgi stacks. Some grains appeared over secretion granules (Fig. 9).

At 45 and 60 min after injection, fewer grains were seen over the Golgi apparatus, while almost none were present over the rER. The number of grains over secretion granules seemed to diminish. At these and earlier times, a few grains were still seen over mitochondria, plasma membrane, and nucleus.

Counts of the total number of silver grains in the electron microscope (Fig. 10; dotted line at top) followed the same pattern as observed in the light microscope (Table I) with a maximum at 10 min and a decrease to 42% of the peak at 60 min. Counts over individual organelles revealed that the number of silver grains over the rER was high at the earliest interval but decreased rapidly thereafter. Meanwhile, the number of grains over the Golgi apparatus, which was very low at first, increased to reach a peak by 10 min and eventually decreased. The secretion granules showed a small but definite peak of labeling at 20 min and a slow decrease thereafter (Fig. 10).
Using the method of Whur et al. (36) (Table III B) to eliminate those organelles which might be labeled by chance revealed the rER as the only significantly labeled organelle at the earliest interval. With time, the rER count became negligible, while the Golgi uptake became prominent. Secretion granules and plasma membrane showed a significant count between 20 and 60 min.

**Galactose-³H Experiment**

In light microscope radioautographs, the reaction over the parenchyma of the parathyroid gland after injection of tritium-labeled galactose was distributed randomly with a peak count at 10 min (Table II).

The electron microscope radioautographs at 2 min after injection of galactose-³H (Fig. 11) showed silver grains almost exclusively localized over Golgi stacks, with few over rER and plasma membrane.

At 10 min (Fig. 12), besides the accumulation of grains over the Golgi apparatus, a few were observed not only over rER and plasma membrane, but also over secretion granules. At 20 and 30 min after injection of galactose-³H, the labeling of the Golgi apparatus declined further. Silver grains remained present over secretion granules at 20 but not at 30 min.

Grain counts (Fig. 13) confirmed that the Golgi apparatus showed a peak of uptake at 2 min followed by a steady decline and that the secretion granules were unlabeled at first, but reached a small peak of labeling at 20 min before losing label by 30 min.

Identification of significant counts by the method of Whur et al. (36) (Table III C) showed none over rER and mitochondria at any time interval. The count over the Golgi apparatus at 2 min after galactose-³H injection was highly significant, but decreased rapidly with time. A significant count over secretion granules was observed at the 10- and particularly 20-min intervals. The plasma membrane showed a significant count at 30 min.

**DISCUSSION**

The cells of a given parathyroid gland have been assumed to be in different functional states, with some of them active and others not (4, 8, 9, 14, 22). However, silver grains appeared uniformly distributed in light microscope radioautographs following tyrosine-³H or galactose-³H injection. Hence, with regard to the uptake of these substances into protein or glycoprotein, all cells should be in about the same state of activity.

**Production of Protein**

The short pulse of tyrosine-³H (Fig. 1) allowed a clear-cut distinction between the 2-min pattern when silver grains were mostly over rER (Figs. 6, 7) and the 5-min pattern when silver grains were often over the Golgi apparatus (Fig. 8). The 2-minute pattern indicated that the site of protein synthesis was the rER. Presumably, as in other cells, protein synthesis took place in relation to ribosomes (39–46).

Between 2 and 10 min after tyrosine-³H injection, there was a sharp drop in the radioactivity of the rER, while the total radioactivity did not decrease. Meanwhile, that of the Golgi apparatus increased, suggesting that the newly synthesized...
Figure 8 5 min after injection of tyrosine-3H. The Golgi stacks (G) are overlaid by silver grains (arrows), indicating that the radioactivity present in the rough endoplasmic reticulum (rER) at 2 min has now migrated to the Golgi apparatus. N, nucleus; L, dense body. X 30,000.

Figure 9 20 min after injection of tyrosine-3H. Radioactivity appears in secretion granules (s). The Golgi apparatus (G) still contains label. N, nucleus; d, desmosome. X 30,000.
protein had migrated from rER to Golgi apparatus. Soon thereafter, the Golgi radioactivity in turn decreased (Table III B; Fig. 10), whereas there was some increase in the radioactivity of secretion granules, a fact emphasized by expressing the results in terms of concentration (Fig. 14). Indeed, morphological observations showed secretion granules arising in the Golgi apparatus (2, 8, 9, 12, 14, 19, 20). The eventual decrease in the radioactivity of secretion granules suggested that their content was released outside the cell.

When total radioactivity was plotted against the log of time starting from 10 min after injection, the half-life of the labeled proteins in both light and electron microscope radioautographs was found to be about 45 min. Such short half-life supports the hypothesis that much of the newly synthesized protein constitutes the parathyroid hormone, since Sherwood et al. (47) have provided evidence for a rapid turnover of this hormone, with quick response of the gland to fluctuations in blood calcium.

Production of Carbohydrate

It has been shown that, following an intravenous injection of galactose-3H, galactose is cleared from the blood in a few minutes (48) and therefore the pulse of labeled galactose available to the tissues was very short. At 2 min after galactose-3H injection, the only significant uptake of label was in the Golgi apparatus. In preliminary work in this Department by A. Haddad, a similar uptake of fucose-3H label was observed in parathyroid cells. The incorporation of these two sugars indicated that a glycoprotein is being elaborated by these cells. It is already known that the Golgi apparatus intervenes in the synthesis of glycoprotein (36, 46, 48-51), probably at the end of the stepwise building of their carbohydrate side chains (48).

With time, the radioactivity of the Golgi apparatus fell (Fig. 13), while that of secretion granules increased to a peak at 20 min and declined later. The label would thus migrate from the Golgi apparatus into secretion granules, which in turn would excrete their content outside the cell.
Presumably then, the secretion granules carry the newly synthesized glycoprotein from Golgi apparatus to the outside.

The periodic acid–silver methenamine technique for glycoprotein showed moderate or weak staining of the membrane of secretion granules, whereas dense and multivesicular bodies were deeply stained (Bodak-Gyovai, unpublished). However, dense and multivesicular bodies were seldom labeled, whereas secretion granules often were. It is presumed that the labeled glycoprotein carried by these granules is associated with their membrane.

Relation of Protein and Carbohydrate

The most likely interpretation of the results was that the pathway of the galactose label was from Golgi apparatus into secretion granules and from there to the outside of the cells. The pathway would be the same as that of the tyrosine label from the Golgi apparatus onward. It might be objected that the decrease in the Golgi content of galactose label with time (Fig. 13) was more precipitous than that of tyrosine label (Fig. 10). This difference may simply be due to different availability of the two labels. The labeled galactose is pro-
Figure 14  Relative concentration of silver grains over parathyroid cells at various times after injection of tyrosine-3H. Three peaks are distinguishable: rER at 2 min, Golgi at 10 min, and secretion granules at 20-30 min.

vided to the Golgi apparatus by the blood, but only during a short time, for its half-life in the blood estimated from data obtained soon after injection (48) is 3.5 min. The tyrosine label is provided to the Golgi apparatus by the rER over a longer period of time, for the half-life in the rER (calculated after the 10-min interval) is 23 min. Hence, the difference in the Golgi slopes in Figs. 10 and 13 should not be interpreted as necessarily indicating a different behavior of the two labels.

Under these conditions, the first possibility which came to mind was that the two labels were both taken into the parathyroid hormone, which would itself be a glycoprotein. It is realized that the hormone extracted from the glands of several species was found to be a pure polypeptide (23, 27, 52). Nevertheless, a carbohydrate moiety might be lost in the course of the extraction procedure. Sherwood et al. (53) have shown that a precursor of higher molecular weight than the parathyroid hormone, a pro-parathyroid hormone, is present in bovine parathyroid tissue. In addition, the hormone extracted from the gland is radioimmunologically different from the hormone found either in serum or in a culture medium in which bovine or human parathyroid tissue is grown (53, 54). It is not impossible, therefore, that this difference may be due to the intracellular hormone being associated with carbohydrate which would be cleaved off following release from the cell.

Another interpretation is suggested by the possibility that the labeled glycoprotein is carried by the membrane of secretion granules, as mentioned above. The hormone itself would then be a pure polypeptide carried within the secretion granules. As the granules release their content outside the cell by exocytosis, their membrane would fuse with the plasma membrane of the cell and provide it with surface glycoprotein. Let us recall that the plasma membrane was significantly labeled 30 min after galactose-6H injection (Table III C), a result again suggesting that the destination of the glycoprotein was the cell surface. Late labeling of the membrane was also observed after tyrosine-6H (Table III B), possibly indicating passage of some of its radioactivity into the protein moiety of the surface glycoprotein. Recently, the passage of label from Golgi apparatus to cell surface was reported in the rat duodenal epithelium after injection of galactose-6H (50) or fucose-6H (51) and was interpreted as indicating continuous production of the surface glycoprotein making up the so-called cell coat. The present data suggest that cell coat material may also be produced continuously in the Golgi apparatus of parathyroid cells and carried to their surface as membrane of secretion granules.

In conclusion, the parathyroid cell of the rat synthesizes and releases protein material which is presumed to be mainly the parathyroid hormone. There is also elaboration of carbohydrate material which may be associated with the hormone in the cell or constitute a distinct glycoprotein, which may be that of the cell coat.

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REFERENCES

1. LEVER, J. D. 1957. Fine structural appearance in the rat parathyroid. J. Anat. 91:173.
2. DAVIS, R., and A. C. ENDELS. 1961. Light and electron microscope studies on the parathyroid gland. In The parathyroids. R. O. Greppe and R. V. Talmadge, editors, Charles C Thomas, Publisher, Springfield. Ill. 76.
3. ROTH, S. I., and L. G. RAOZ. 1964. Effect of calcium concentration on the ultrastructure of rat parathyroid in organ culture. Lab. Invest. 13:531.
4. ROTH, S. I., and L. G. RASZ. 1966. The course and reversibility of the calcium effect on the ultrastructure of the rat parathyroid gland in organ culture. Lab. Invest. 15:1187.
5. MAZZOCCHI, G., V. MANEGHELLI, and M. T. SERAFINI. 1967. The fine structure of the parathyroid glands in the normal, the rachitic and the bilaterally nephrectomized rat with special interest to their secretory cycle. Acta Anat. 68:550.
6. ROTH, S. I. 1962. Pathology of the parathyroids in hyperparathyroidism. Discussion of recent advances in the anatomy and pathology of the parathyroid glands. Arch. Pathol. 73:949.
7. ROTH, S. I., and B. L. MUNGER. 1962. The cytology of the adenomatous, atrophic and hyperplastic parathyroid glands of man. A light and electron microscopic study. Virchows Arch. Abt. A:Pathol. Anat. 335:393.
8. MUNGER, B. L., and S. I. ROTH. 1963. The cytology of the normal parathyroid glands of man and Virginia deer. A light and electron microscopic study with morphologic evidence of secretory activity. J. Cell Biol. 16:379.
9. CAPEN, C. C., A. KOESTNER, and C. R. COLE. 1965 a. The ultrastructure and histochemistry of normal parathyroid glands of pregnant and non-pregnant cows. Lab. Invest. 14:1673.
10. CAPEN, C. C., A. KOESTNER, and C. R. COLE. 1965 b. The ultrastructure, histopathology, and histochemistry of the parathyroid glands of pregnant and non-pregnant cows fed a high level of vitamin D. Lab. Invest. 14:1809.
11. FUJIMOTO, Y., K. MATSUKAWA, H. INUBOSHI, M. NAKAMASU, H. SATOH, and S. YAMAGIWA. 1967. Electron microscopic observations of the equine parathyroid glands with particular reference to those of equine osteodystrophia fibrosa. Jap. J. Vet. Res. 15:537.
12. MELSON, G. L. 1966. Ferric glycerophosphate-induced hyperplasia of the rabbit parathyroid gland. An ultrastructural study. Lab. Invest. 15: 818.
13. TRIER, J. S. 1958. The fine structure of the parathyroid gland. J. Biophys. Biochem. Cytol. 4:13.
14. NAKAGAMI, K. 1965. Comparative electron microscopic studies of the parathyroid gland. I. Fine structure of monkey and dog parathyroid glands. Arch. Histol. Jap. 25:435.
15. MIZUOCCHI, Y. 1958. Histological studies on parathyroid. III. Electron microscopic observations on parathyroid of a dog. Med. J. Kagoshima Univ. 10:1079.
16. CAPEN, C. C., and G. N. ROWLAND. 1968. The ultrastructure of the parathyroid glands of young cats. Anat. Rec. 162:237.
17. KAYSER, L., A. PETROVIC, and A. PORTE. 1961. Variations ultrastructurales de la parathyroide du hamster ordinaire (Cricetus cricetus) au cours du cycle saisonnier. C. R. Soc. Biol. Paris. 155:2178.
18. HARA, J., and I. NAKAGAMI. 1964. Electron microscopic study of the parathyroid gland of the mouse. Nagoya J. Med. Sci. 26:119.
19. NAKAGAMI, K. 1967. Comparative electron microscopic studies of the parathyroid gland. II. Fine structure of the parathyroid gland of the normal and the calcium chloride treated mouse. Arch. Histol. Jap. 28:185.
20. STOECKEL, M. E., and A. PORTE. 1966 a. Observations ultrastructurales sur la parathyroide de souris. I. Etude chez la souris normale. Z. Zellforsch. Mikrosk. Anat. 73:488.
21. STOECKEL, M. E., and A. PORTE. 1966 b. Observations ultrastructurales sur la parathyroide de souris. II. Etude experimentale. Z. Zellforsch. Mikrosk. Anat. 73:503.
22. FEYTER, A. W., and C. C. CAPEN. 1970. The ultrastructure of the parathyroid glands on young pigs. Acta Anat. 75:359.
23. HAWKER, G. D., J. D. GLASS, and H. RASMUSSEN. 1966. Further studies on the isolation and characterization of parathyroid polypeptides. Biochem. J. 3:534.
24. GREULICH, R. C., and C. P. LEBLOND. 1953. Radioautographic visualization of radiocarbon in the organs and tissues of newborn rats following administration of C14-labeled bicarbonate. Anat. Rec. 115:539.
25. POTCHEN, E. J. 1963. Isotopic labeling of the rat parathyroid as demonstrated by autoradiography. J. Nucl. Med. 4:480.
26. RASMUSSEN, H., and L. C. CRAIG, 1961, Isolation and characterization of bovine parathyroid hormone. J. Biol. Chem. 236:759.
27. POTTS, J. T., G. D. AXERBACH, and L. M. SHERWOOD. 1966. Parathyroid hormone. Chemical properties and structural requirements for biological and immunological activity. Recent Progr. Hormone Res. 22:101.
28. EYLAR, E. 1965. On the biological role of glycoproteins. J. Theor. Biol. 10:89.
29. Peters, Th., Jr., and C. A. Ashley. 1967. An artefact in radioautography due to binding of free amino acids to tissues by fixatives. J. Cell Biol. 38:53.

30. Bergerson, M., and B. Droz. 1968. Analyse critique des conditions de fixation et de préparations des tissus pour la détection radioautographique des protéines néotéromées, en microscopie électronique. J. Microsc. 7:51.

31. Kopriwa, B. M., and C. P. Leblond. 1962. Improvements in the coating technique of radioautography. J. Histochem. Cytom. 10:269.

32. Salpeter, M. M., and L. Bachmann. 1965. Assessment of technical steps in electron microscope autoradiography. In The Use of Radioautography in Investigating Protein Synthesis. C. P. Leblond and Katherine B. Warren, editors. Academic Press Inc., New York. 4:43.

33. Granboulan, P. 1965. Comparison of emulsions and techniques in electron microscope radioautography. In The Use of Radioautography in Investigating Protein Synthesis. C. P. Leblond and Katherine B. Warren, editors. Academic Press Inc., New York. 4:23.

34. Kopriwa, Beatrix Markus. 1967 b. The influence of development on the number and appearance of silver grains in electron microscope radioautography. In The Use of Radioautography in Investigating Protein Synthesis. C. P. Leblond and Katherine B. Warren, editors. Academic Press Inc., New York. 4:15.

35. Kopriwa, B. M. 1967 a. A semiautomatic instrument for the radioautographic coating technique. J. Histochem. Cytom. 14:923.

36. Chalkley, H. W. 1943. Method for the quantitative morphologic analysis of tissues. J. Nat. Cancer Inst. 4:47.

37. Revel, J. P., and E. D. Hay. 1963. An autoradiographic and electron microscopic study of collagen synthesis in differentiating cartilage. Z. Zellforsch. Mikrosk. Anat. 61:110.

38. Warshawsky, H., C. P. Leblond, and B. Droz. 1963. Synthesis and migration of proteins in the cells of the exocrine pancreas as revealed by specific activity determination from radioautographs. J. Cell Biol. 16:1.

39. Nadler, N. J., B. A. Young, C. P. Leblond, and B. Mitmaker. 1964. Elaboration of thyroglobulin in the thyroid follicle. Endocrinology. 74:333.

40. Van Heyningen, H. E. 1964. Secretion of protein by the acinar cells of the rat pancreas, as studied by electron microscopic radioautography. Anat. Rec. 148:345.

41. Caro, L. G., and G. E. Palade. 1964. Protein synthesis, storage and discharge in the pancreatic exocrine cell. An autoradiographic study. J. Cell Biol. 26:473.

42. Wellings, S. R., and J. R. Philp. 1964. The function of the Golgi apparatus in lactating cells of the BALB/c Crj mouse. An electron microscopic and autoradiographic study. Z. Zellforsch. Mikrosk. Anat. 61:871.

43. Leblond, C. P., and Katherine B. Warren, editors. 1965. The Use of Radioautography in Investigating Protein Synthesis. Academic Press Inc., New York. 4.

44. Young, R. W., and B. Droz. 1968. The renewal of protein in retinal rods and cones. J. Cell Biol. 39:169.

45. Haddad, A., Meredith D. Smith, Annette Herscovics, C. J. Nadler, and C. P. Leblond. 1971. Radioautographic study of in vivo and in vitro incorporation of fucose-3H into thyroglobulin by rat thyroid follicular cells. J. Cell Biol. 49:836.

46. Sheppard, L. M., G. P. Mayer, C. F. Ramberg, Jr., D. S. Kronfeld, G. D. Aeberbach, and J. T. Potts, Jr. 1968. Regulation of parathyroid hormone secretion: proportional control by calcium, lack of effect of phosphate. Endocrinology. 83:1043.

47. Weinstock, A., and C. P. Leblond. 1971. Elaboration of the matrix glycoprotein of enamel by the acinar cells of the rat pancreas, as revealed by radioautography after galactose-3H injection. J. Cell Biol. 51:28.

48. Haddad, A., Meredith D. Smith, Annette Herscovics, N. J. Nadler, and C. P. Leblond. 1971. Radioautographic study of in vivo and in vitro incorporation of fucose-3H into thyroglobulin by rat thyroid follicular cells. J. Cell Biol. 49:856.

49. Bennett, G. 1970. Migration of glycoprotein from Golgi apparatus to cell coat in the columnar cells of the duodenal intestine. J. Cell Biol. 45:668.

50. Bennett, G., and C. P. Leblond. 1970. Formation of cell coat material for the whole surface of columnar cells in the rat small intestine, as visualized by radioautography using 3H-L-fucose. J. Cell Biol. 46:409.

51. Littlefield, E. T., and C. D. Hawker. 1967. Extraction, purification and partial characterization of porcine parathyroid hormone. Endocrinology. 81:261.

52. Sheppard, L. M., J. S. Rodman, and W. B. Lundberg. 1970. Evidence for a precursor to circulating parathyroid hormone. Proc. Nat. Acad. Sci. U.S.A. 67:1631.

53. Arnaud, Claude D., Hang S. Tsao, and Susan B. Oldham. 1970. Native human parathyroid hormone: an immunochemical investigation. Proc. Nat. Acad. Sci. U.S.A. 67:415.