3. Balls, A. K., Ryan, C. A., *J. Biol. Chem.*, 1963, v238, 2976.
4. Jansen, E. F., Nutting, M.D.F., Jang, R., Balls, A. K., *ibid.*, 1949, v179, 189, 195-6.

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Autoradiographic Localization of Iodide^{125} in the Thyroid Epithelial Cell. (29035)

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Autoradiography has been employed to investigate the localization of inorganic iodide^{131} within the thyroid follicle in several species(1,2). These studies were performed in animals in which the incorporation of iodide^{131} into thyroidal protein-bound iodine^{131} (PBI^{131}) was inhibited by administration of a goitrogen and at a time when thyroidal iodide^{131} was almost equilibrated with serum iodide^{131}. Radioiodide was localized primarily in the follicular lumen and cellular radioiodide concentration was greater than that in blood vessels. It appeared possible that blood vessel and cellular radioiodide concentrations may not have been appreciably different *in vivo*, and that the excess cellular radioiodide may have arisen by diffusion of luminal radioiodide after excision of the thyroid gland. Therefore it seemed possible that the iodide concentrating mechanism might be localized at the apical end of the epithelial cell. Recent studies by Tong *et al*(3), however, have demonstrated that isolated thyroid epithelial cells are able to concentrate radioiodide. We have made systematic investigations of the autoradiographic localization of iodide^{125} in the intact thyroid follicle *in vivo* and here report conditions in which radioiodide is more concentrated in the follicular epithelium than in the follicular lumen.

Methods. Male C_3H mice, 3 to 5 months of age and fed Purina Laboratory Chow since weaning, were used in these studies. Where indicated animals were given 2 mg of propylthiouracil in 0.04 N NaOH by subcutaneous injection 30 minutes before iodide^{125} in order to inhibit the formation of thyroidal PBI^{125}. Under pentobarbital anesthesia mice were given 100-200 μc of iodide^{125} by intraperitoneal injection. Five minutes later glands were quickly removed while still attached to the trachea and plunged into isopentane cooled to about —160°C by liquid nitrogen. Thyroids were dried at —45°C, paraffin embedded in vacuum and sectioned at 6 μ. Sections were mounted permanently in contact with the emulsion on glass slides covered with AR-10 autoradiographic stripping film. After suitable exposure, the "integrated autoradiographs" were developed, fixed and stained with Nuclear Fast Red by methods to be described in detail elsewhere. Control studies with non-radioactive thyroids showed that histologic sections did not cause chemical induction of a latent image and did not significantly modify a uniform latent image produced by soft X-rays.

Results. Organic binding of radioiodide blocked. In about 60% of the 20 thyroids examined at least a few follicles in each gland had autoradiographic images primarily over the epithelial cells 5 minutes after iodide^{125} administration (Fig. 1, a and b). Approximately 20% of the thyroids contained autoradiographic rings associated with a third or more of the thyroid follicles. In the remaining follicles the autoradiographic grain density over the lumen was at least equal to the grain density over the epithelium. There was a much lower density of grains over blood vessels and parathyroid glands. At later times after radioiodide injection our findings were similar to those previously reported by others(1,2).

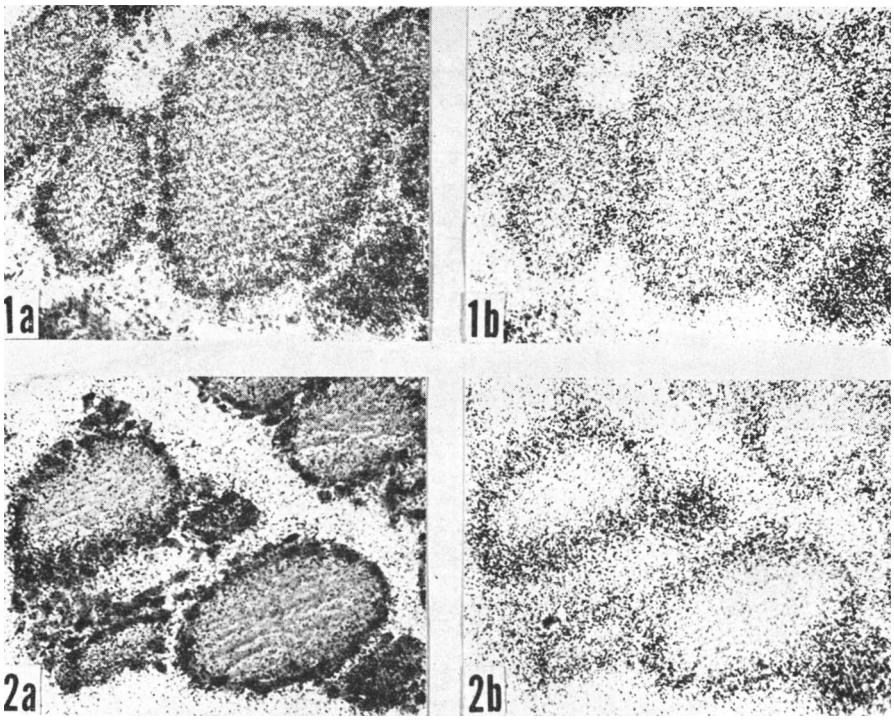

FIG. 1. Autoradiograph of iodide^{125} in thyroid of a mouse treated with propylthiouracil to inhibit PBI^{125} formation. Iodide^{125} was administered 5 min before thyroid was excised. (a) Photomicrograph using green filter to visualize histologic details relative to silver grains. Microscope focused on silver grains. Note red cells in blood vessels. × 300. (b) Same field as Fig. 1 (a) using red filter. Comparison of the grain pattern in Fig. 1 (a) and 1 (b) with the position of nuclei in Fig. 1 (a) demonstrates that the autoradiographic rings lie directly over the follicular epithelium. Grain density is lower over lumens and blood vessels. Granular image over the red cells is caused largely by incomplete extinction of red cells by filter. × 300.

FIG. 2. Autoradiograph of iodide^{125} in thyroid of a mouse 5 min after iodide^{125} administration. PBI^{125} formation permitted. (a) Photomicrograph using green filter to visualize histologic details as in Fig. 1 (a). × 300. (b) Same area as Fig. 2 (a) using red filter. Autoradiographic rings lie over follicular epithelial cells. No image is apparent from PBI^{125}. × 300.

Organic binding of radioiodide permitted. It is difficult to study the localization of radioiodide in the thyroid gland that can form PBI since radioiodide is generally a small fraction of the total thyroidal radioiodine. However, at early times after radioiodide administration the concentration of radioiodide is several times that of protein-bound radioiodine(4). When the amount of PBI^{125} is relatively very small it makes a negligible contribution to the autoradiograph and does not interfere with the visualization of inorganic iodide^{125}. In the thyroid gland of the animals in which organic binding was allowed to proceed the autoradiographic image was primarily over the cell 5 minutes after intraperitoneal injection of iodide^{125} (Fig. 2, a and b). Fig. 2, a and b, were selected from an area of a gland with very little PBI^{125} to avoid obscuring the localization of the iodide^{125}. Autoradiography of thyroids fixed in Bouin's fluid from similarly treated animals revealed only a small amount of PBI^{125} localized in the lumen at the periphery of the colloid. Sections of frozen-dried thyroids which were methanol fixed and extracted to remove the iodide^{125} yielded no autoradiographic image over the cells but only image over the lumen.

Discussion. Concentration of iodide by the thyroid follicle has been considered to be due to the properties of a limiting membrane, *i.e.*, the basal or apical membrane of the epithelial cell. If this be the case, then the localization

of considerable concentrations of radioiodide in the epithelium of the binding and of the propylthiouracil-blocked thyroid gland indicates that an iodide concentrating mechanism is localized at the basal end of the cell. The persistence of the autoradiographic ring for at least 5 minutes after iodide^{125} administration indicates that the apical membrane can limit the transfer of iodide from the cell to the lumen. It is clear that since the epithelial radioiodide concentration is much greater than the concentration in the lumen most of the cellular radioiodide did not arise from diffusion from the lumen.

In propylthiouracil-treated animals variation in the occurrence of rings from follicle to follicle and from animal to animal may be a reflection of variation in metabolic activity of the glands examined. Experiments to be reported elsewhere have demonstrated that in hypophysectomized mice there were autoradiographic rings associated with almost all follicles in all animals studied 10 minutes after radioiodide injection whereas in animals fed a low iodine diet for 2 weeks autoradiographic rings were rarely seen 4 minutes after radioiodide injection.

Summary. At short time intervals after injection of iodide^{125}, the concentration of inorganic iodide^{125} in the thyroid epithelial cells of some follicles was much higher than in the lumen of the follicle or in blood vessels as demonstrated by autoradiographic methods. This finding in both binding and propylthiouracil-blocked thyroid glands suggests that the basal cell membrane is responsible for iodide concentration. The persistence of an autoradiographic image over the follicular cells for at least 5 minutes after iodide^{125} injection suggests that the apical cell membrane exerts some control over the passage of radioiodide from the cell into the follicular lumen.

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1. Pitt-Rivers, R., Trotter, W. R., *Lancet,* 1953, v265, 918.

2. Doniach, I., Logothetopoulos, J. H., *J. Endocrinol.,* 1955, v13, 70.

3. Tong, W., Kerkof, P., Chaikoff, I. L., *Biochim. Biophys. Acta,* 1962, v60, 1.

4. Wollman, S. H., Reed, F. E., *Am. J. Physiol.,* 1962, v202, 182.

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Effects of Sulfaguanidine on Rat Submaxillary Gland. (29036)

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Thyroidectomy or goitrogen administration induces reduction in relative and absolute gland weights and atrophic degranulation of granular tubules in the rat submaxillary gland(1-3). Sulfonamides are unique goitrogens in that their antithyroid action is potentiated by iodides(4). This study reports the effects of sulfaguanidine upon weight and morphology of the rat submaxillary gland.

Methods. Walter Reed strain male albino rats weighing 230-280 g (10-11 weeks old) were used in 28-day experiments. In 2- and 4-month experiments, animals weighed initially 140-160 g (8 weeks old). Basal diet consisted of ground D&G rat biscuits* (iodine content, 800 μg/kg) and tap water *ad lib.* This diet was altered by addition of 2% sulfaguanidine (SG)† or 0.5% NaI, singly or combined, and used for appropriate groups (Tables II and III). Surgical thyroidectomies (Tx) were performed on certain animals under ether anesthesia on the first day of the experiment. Animals were weighed weekly and at sacrifice. In all experiments

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