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Separation of the "Colloid" from Living Thyroid Gland by Means of Centrifugal Force.

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Following the lead of E. P. Lyon¹ and others, in some previous work I have shown that it is possible by means of a centrifugal force of about 3,000 times gravity to cause separation of heterogeneously dispersed materials inside living cells into distinct layers, depending on their density, without killing the cells, even though they were kept in the centrifuge for a week. In later centrifuge experiments^{2, 3} I separated crystals appearing homogeneous in the polarizing microscope and isolated them for chemical analysis. When these crystals were treated with alcohol they were decomposed into a phospholipin and a vitellin. Cavett, Rice and McClendon⁴ reported that thyroglobulin treated with acetone and ether (as a substitute for alcohol, which denatured it) lost weight (increased in iodine percentage), which I interpreted as due to splitting off of some lipin. To test this hypothesis some method of getting thyroglobulin from thyroid without contamination with cell proteins was needed.

Thyroglobulin, the protein of the thyroid gland, is present in the sol or gel known as "colloid", which is contained in the thyroid follicles. The follicles containing "colloid" are of a spheroidal shape formed by a single layer of flattened or cuboidal cells. Since spaces between these cells have been reported by cytologists it occurred to

¹ Lyon, E. P., *J. Exp. Zool.*, 1909, **6**, 269.

² McClendon, J. F., *Arch. Entwicklungsmech.*, 1909, **27**, 247.

³ McClendon, J. F., *Am. J. Physiol.*, 1908, **23**, 460; 1909, **25**, 195.

⁴ Cavett, Rice and McClendon, *J. Biol. Chem.*, 1935, **110**, 673.

me that by the use of a sufficiently high centrifugal field, the "colloid" might be removed from the follicle.

When a portion of rabbit thyroid tissue was subjected to the centrifugal force of 160,000 to 200,000 times gravity in the ultracentrifuge at Fysikalisk-Kemiska Institutionen, Upsala, a fluid separated, forming a layer above the tissue. If the centrifugal force of 100,000 times gravity is prolonged, one can observe the refraction of the boundary of sedimenting protein. The sedimentation velocity of this protein was obtained through an ultracentrifugal study of a diluted portion of the fluid and was found to correspond to the value previously obtained at this laboratory for thyroglobulin.^{5, 6}

The separated fluid contained a small amount of hemoglobin which was evident from its color. When a portion of a rabbit gland perfused with saline was subjected to the ultracentrifugal field a fluid was obtained which was apparently free from hemoglobin. Microscopic sections showed that many of the follicles had flattened during the procedure. Thyroglobulin was also obtained from hog and human thyroid tissue by centrifugal force.

If the molecules of thyroglobulin passed through the cells on their way out of the follicle they would pass in a centrifugal direction, whereas we found the thyroglobulin passed out in a centripetal direction. Therefore it was not the thyroglobulin that moved but the cells that were acted on by the centrifugal force and precipitated as units. If they were cemented together in continuous sacks they would be buoyed up by the colloid. Therefore there are spaces between the cells.

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⁵ Heidelberger, M., and Svedberg, T., *Science*, 1934, **80**, 414.

⁶ Heidelberger, M., and Pedersen, K., *J. Gen. Physiol.*, 1935, **19**, 95.