Staining of Thyroid Gland with Ferritin-Conjugated Antithyroglobulin (No. 3)

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Recently the ferritin-conjugated antibody method has been applied in a increasing number to intercellular localization of various antigenetic materials. In this report, the ferritin-conjugated antibody method, using the antibody to thyroglobulin, was utilized in an attempt to investigate the site of production and resorption of thyroglobulin in the organelles of thyroid cells.

When bovine thyroid cells after being fixed in glutaraldehyde was incubated in ferritin-conjugated antibody solution, most of ferritin labels were shown to be distributed in the intercellular spaces and only a few inside of the cells. This may indicate that in these intercellular spaces alone is there a substance identical to thyroglobulin in its antigenicity or the cell membrane is not permeable to this conjugate and prevent it from intercellular penetration.

In considering the latter event, we treated fixed thyroid specimens, before their incubation in the conjugate, either with surface-active agents such as triton X-100, deoxycholate, butanol or with sonic vibrations or freezing.

The results after these treatment are summarized in the following table.

TREATMENT		CHANGES OF ULTRASTRUCTURES	LOCALIZATION OF FERRITIN PARTICLES
Triton X-100	0.5% 20min.	few changes	A number of ferritin particles were observed in the cytoplasm, especially on the endoplasmic reticulum.
Freezing	30min.	Plasmamembranes which had been torn, scattered near the cells	
D.O.C. (Deoxycholate)	0.5% 30min.	Blurring of the intracellular ultrastructures.	A few ferritin particles were observed on the endoplasmic reticulum and membranes of the mitochondrion and nucleus when the cell was severely ruptured.
Butanol	20% 30min.	Blurring of the intracellular ultrastructures.	A few ferritin particles were observed in intracellular spaces, but none was found in the cytoplasm.
Sonic vibrations	9KC 30min.	The endoplasmic reticulum was in many places deformed to small vesicles.	No ferritin particles

When bovine thyroid tissue was fractionated by differential ultracentrifugation and fractions thus obtained were treated as above mentioned, a great number of ferritin particles were found on the microsomal fraction and a few on the mitochondrial fraction.

These two fractions, when tested by hemagglutination-inhibition test, inhibited passive hemagglutination of red cells sensitized with thyroglobulin, the microsomal fraction, however, showing by far more the ability; the ratio in concentration of thyroglobulin, as indicated by this test, among subcellular fractions was as follows. mitochondrial fraction: microsomal fraction: the others; 1 : 2⁴ : 2¹⁰.

And the number of ferritin particles present in a unit area of electron micrographs from both fractions was calculated, to obtain the next ratio. mitochondrial fraction: microsomal fraction; 1 : 16.

From the above results combined, thyroglobulin present in the bovine thyroid cells may be mainly associated with the endoplasmic reticulum and the intercellular space is a probable pathway of thyroglobulin into or out of follicular colloid material.

But in this experiment intracellular colloid droplets with ferritin label could not be confirmed.

Histochemistry of Glycogen Metabolism in the Human Placental Tissue (I)

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It has been known that the substance transfer in the placental tissue is not made by simple diffusion from mother to fetus, but should be carried out by some active process with energy-requiring process. The session on the histochemistry of the placenta was opened by Villee who listed the biochemical functions currently ascribed to the placenta. The storage, transport, and synthesis of a variety of substances, and a great number of the enzymatic reactions of intermediary metabolism were investigated. The active transfer of substances across the human placenta appeared to suggest its function.

From this point, the distribution and localization of enzymes, particularly alkaline glycerophosphatase were histochemically investigated, and glycogen contents was also observed by periodic acid Schiff stain, using the normal human placenta in the various gestational ages. Alkaline glycerophosphatase in trophoblastic villi was demonstrated mainly in trophoblastic cells and cell-islands at the early gestation ages, but it was moderately stained in proliferative bud to trophoblastic cells. The activity was gradually increased with the gestation process. On the contrary, the phosphorylase activity was strongly demonstrable in the trophoblastic cell in 4th and 5th month of the gestation. Thereafter it