Iodine-125 Distribution between Follicular Colloid and Colloid Droplets in Mouse Thyroid Gland

Abstract. *Radioautographs of tissue from the thyroid gland prepared 24 hours after the administration of iodine-125 showed that the concentration of radioactivity in intracellular colloid droplets was the same as that in the follicular colloid. This observation indicates that colloid droplets are due to phagocytosis of small portions of follicular colloid.*

For many years thyrotrophic hormone has been known to cause a rapid increase in the number of intracellular "colloid droplets" (*1*). Although the staining and histochemical properties of these droplets are similar to those of follicular colloid (*2*) there is a divergence of opinion as to their origin. Morphologic evidence obtained by electron microscopy has been interpreted to show that colloid droplets are newly formed thyroglobulin and are secreted from the apex of the cell into the follicular space (*3*). However, histochemical studies combined with light-microscopy autoradiographic studies have indicated that "colloid droplets" are bits of follicular colloid incorporated into the thyroid cells, and proteolysis of the thyroglobulin within colloid droplets accompanied by the release of thyroxine has been considered one of the possible mechanisms of thyroid hormone release (*4*). We have studied the distribution of I^{125} between colloid droplets and follicular colloid using combined techniques of autoradiography and electron microscopy, and report here the presence of iodinated protein in both locations. Equal concentrations of the labeled iodine were found in the two locations. Such evidence indicates that colloid droplets originate from follicular colloid.

Iodine-125 (20 μc) was injected into the peritoneum of adult male mice (Charles River CD-1 albino). Twenty-four hours later they were divided into two groups. The animals in group 1 were anesthetized and their thyroid glands removed rapidly, fixed in phosphate-buffered (*p*H 7.2) osmium tetroxide (2 percent) solution, dehydrated in alcohol, and embedded in Epon 812. Animals in group 2 were given 0.5 units of bovine thyrotrophic hormone (Armour Thytropar) intraperitoneally and 15 minutes later their thyroid glands were excised, fixed, and embedded as for group 1. Thin sections for electron microscopy were coated with Ilford L-4 emulsion (*5*), exposed for 13 to 16 days, developed, and fixed. The emulsion was removed by immersion of the grids in 0.05*N* sodium hydroxide for 20 minutes. The sections were then stained with alcoholic uranyl acetate and examined in an electron microscope (*6*).

Table 1. Distribution of I^{125} among colloid droplets, follicular colloid, and interfollicular areas (background). Twenty-four hours after the administration of radioactive iodine the animals in group 1 were killed, while those in group 2 were injected with thyrotrophic hormone and then killed 15 minutes later. The ratio of radioactivity in colloid droplets to that in follicular colloid is shown for each group. Area was measured in arbitrary units.

Material	Area (*A*)	No. of grains	No. of grains per 100 × *A*
	Group 1		
Colloid droplets	1,050	66	6.29
Follicular colloid	73,990	3919	5.30
Background	35,407	46	0.13
Ratio of activity	1.19		
	Group 2		
Colloid droplets	4,856	291	5.99
Follicular colloid	72,843	4733	6.50
Background	22,100	7	0.03
Ratio of activity	0.92		

The procedure resulted in electron micrographs with good localization and adequate numbers of grains, and low background grain counts (Fig. 1, *A* and *B*). Twenty-four hours after the administration of the radioactive iodine virtually all of the I^{125} activity was associated with the follicular colloid. Colloid droplets were relatively few but definitely contained I^{125} (Fig. 1*A*). Few grains were found over the cytoplasm of cells unassociated with colloid droplets; the density of such grains by visual

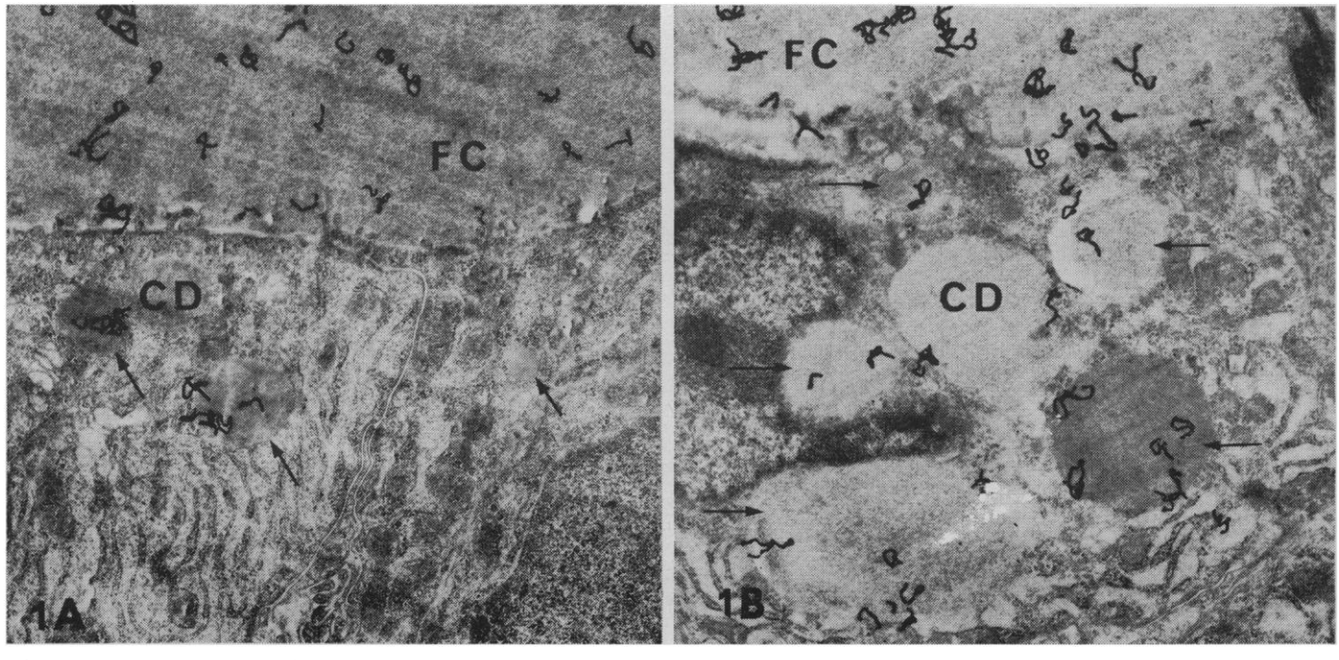

Fig. 1. *A*, An autoradiograph of a cell from the thyroid gland of a normal mouse after the administration of I^{125}. Numerous reduced silver grains are shown over the follicular colloid (*FC*). Four colloid droplets (*CD* and arrows) are seen near the apex of the cell. Several grains are localized over two of the droplets (× 7000). *B*, Large labeled colloid droplets (*CD* and arrows) 15 minutes after stimulation with thyrotrophic hormone. Labeled follicular colloid (*FC*) is at the top (× 7000).

inspection approximated that of background. Administration of thyrotrophic hormone greatly increased the number of colloid droplets and they, too, were clearly labeled (Fig. 1*B*). Quantitative estimations of grain counts were made by photographing every available field at a conveniently fixed magnification (× 4000), enlarging the photographs to 10,000 times, and superimposing on the enlargements a standard grid. The results are shown in Table 1. The number of grains per unit area for colloid droplets and follicular colloid is close to the same value in both groups and results in a ratio of colloid-droplet I^{125} activity to follicular colloid activity that differs insignificantly from unity.

These data show clearly that iodinated protein is present within the confines of the thyroid cell in the form of colloid droplets, and that its activity matches that of iodinated thyroglobulin (follicular colloid) under conditions of physiologic and exogenous stimulation by thyrotrophic hormone. It is not possible to account for the corresponding I^{125} activity in both follicular colloid and colloid droplets in these experiments by any mechanism of formation in which newly synthesized thyroglobulin would be used. We would expect newly formed thyroglobulin to be iodinated to a minimum degree under the conditions of this experiment and to result in a ratio of activities much less than unity. The simplest explanation of these findings is that colloid droplets are derived from follicular colloid in the normal thyroid gland and that follicular colloid is rapidly taken into the cell following exogenous hormonal stimulation. Considerable weight must therefore be given to the observations (*4*) suggesting that fragments of follicular colloid are engulfed by cytoplasmic extensions of the thyroid cell and drawn into the interior. Proteolysis of thyroglobulin and release of thyroxine then may ensue.

WALTER C. BAUER
JOHN S. MEYER
Department of Surgical Pathology and Pathology, Washington University School of Medicine, St. Louis, Missouri 63110

References and Notes

1. M. Grant, *Anat. Record* **46**, 205 (1930); *ibid.* **49**, 373 (1931); E. DeRobertis, *ibid.* **84**, 125 (1942).
2. E. DeRobertis, *Ann. N.Y. Acad. Sci.* **50**, 317 (1949); I. Gersh, *J. Endocrinol.* **6**, 282 (1949).
3. E. Dempsey and R. Peterson, *Endocrinology* **56**, 46 (1955); S. Wissig, *J. Biophys. Biochem. Cytol.* **7**, 419 (1960); *J. Cell Biol.* **16**, 93 (1963).
4. N. Nadler, S. Sarkar, C. Leblond, *Endocrinology* **71**, 120 (1962); S. Wollman and S. Spicer, *Federation Proc.* **20**, 201 (1961).
5. L. Caro, R. Van Tubergen, J. Kolb, *J. Cell Biol.* **15**, 173 (1962).
6. Radio Corporation of America, EMU 3F.
7. Supported by a grant from the John A. Hartford Foundation.

22 June 1964

Genetic Activity in a Heterochromatic Chromosome Segment of the Tomato

Abstract. *The first example of genetic activity within a heterochromatic region of the tomato is provided by the delimitation of* nv *to the long arm of chromosome 9 by means of the induced deficiency method. A close spatial relationship between* nv *and* ah *was established by deficiencies for* ah *in the same arm and by linkage tests between the two genes.*

As part of our research on the genome of the tomato (*Lycopersicon esculentum*), we have attempted to find suitable genetic markers for chromosome 9 and to identify these with cytological landmarks. By means of the trisomic ratio method, *ah* and *wd* were related to this chromosome (*1*). Standard F_2 tests revealed tight linkages (<3 morgans) between the following pairs of genes: *ah-wd, ah-Tm*$_2$, *nv-Tm*$_2$, *ah-pum,* and *ah-nv*. In view of our interest in the last interval, the following pertinent linkage data are presented. Ten recombinants were yielded in a total of 46,577 F_2 segregants of the trans cross (24,793 + +, 9994 + *nv*, 11,780 *ah* +, 10 *ah nv*), corresponding to a crossover value of 3.2 ± 0.6 morgans. The most critical F_3 tests were obtained from the *ah*/*ah* F_2 segregants: eight proven heterozygous for *nv* and 284 homozygous for the normal allele. From the equation

$$\frac{2p}{1-p} = \frac{8 \times 100}{284}$$

1.4 is estimated for the *ah nv* distance.

More definitive information concerning these loci was then sought by means of the induced deficiency method (*2*). For this purpose individuals homozygous for *ms*$_{17}$, *ah, yv,* and *dl* were crossed with var. Red Cherry, which possesses the normal, dominant alleles for these loci. Mature pollen of the Red Cherry was treated with 5000 r of x-rays (generated at 90 kv, filtered with ½ mm aluminum, and delivered at an intensity of 300 r/min). Emasculated, fertile *nv*/*nv* plants were hybridized with irradiated pollen bearing the normal allele in order to produce *nv* deficiencies. A total of 1079 progeny of the former cross included two that were mutant in phenotype only for *ah* (anthocyanin deficiency) and two, subsequently demonstrated to be haploid, that were mutant for all four marker genes. Of the two *ah* mutants, one showed no cytological abnormality, and the other, 63L744-1, had an interstitial deficiency of the long arm of chromosome 9 (9L). A second interstitial deficiency for the *ah* region in 9L, 63L-1850-1, was effected in the progeny of another cross sired by irradiated pollen. Both deficiencies embraced heterochromatic as well as euchromatic regions (Fig. 1) and delimit the *ah* locus to a region including the distal four knobs of the heterochromatic zone and the proximal fourth of the euchromatic.

Of special interest are the cytological features of an *nv* deficiency, 63L1878-1, yielded by *nv* hybridization. Three plants in a total progeny of 1734 showed the *nv* phenotype (patterned chlorophyll deficiency): one haploid, another with no detectable cytological aberration, and the third—a plant with vigor and phenotype comparable with that of nondeficient *nv*—, the aforementioned deficiency heterozygote. The deficiency in this last individual also proved to be interstitial, but with the deleted portion limited entirely to the heterochromatic zone of 9L. The consistent appearance in more than a score of cells of good preparations left no doubt that the treated chromosome of 63L1878-1 is clearly deficient for most of the internal heterochromatin of 9L, but still retains the proximal knob, the two distal ones, and the large euchromatic gap, which is so diagnostic for this chromosome (Figs. 2–4). Pairing is disrupted solely for the deficient region in Fig. 4 and for an additional distal region in Fig. 2, while heterochromatic knobs seem to be nonhomologously associated within the loop in Fig. 3. A somewhat shorter interstitial deficiency for 9L was previously reported (*3*), but it was not related genetically to the corresponding linkage map. The extent of each of the three deficiencies reported here is indicated diagramatically in Fig. 6.

The gene *nv* is the first marker in