# Radioautographic Evidence of $^{3}$H-Tryptophan Incorporation in Secretory Cells of Rat Submandibular Glands

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*Tritiated tryptophan was injected intravenously into male rats, and the submandibular glands were removed at time intervals up to three hours after injection. Grain counts were made on light and electron microscope radioautographs to determine the effects on the amino acid.*

There is a simultaneous increase of both trypsinlike proteolytic activity and the number of granules in the convoluted granular tubules (CGT) of the rat submandibular gland (SMG) during postnatal development.[1] Development and maintenance of the CGT granules and proteolytic activity are partially dependent on sex hormones, pituitary secretions, and thyroxine.[1-4] Recently, five trypsinlike enzymes, one of which (glandulain) is testosterone-induced, and another (salivain) which is a major secretory digestive enzyme, have been isolated from homogenates of rat SMGs and purified.[5]

Because tryptophan is found exclusively in the granules of the CGT, Sreebny and Meyer[6] suggest that CGT cells are responsible for the trypsinlike proteolytic activity. However, because the organelles of CGT cells are not arranged in a pattern similar to patterns of other exocrine cells,[7-10] protein in the granules may be synthesized elsewhere or by an uncommon mechanism.[6] This possibility was recognized by early investigators who found that the CGT, in contrast to other parenchymal cells of the body, accumulated more label at 24 and 36 hours than at 4 hours after injection of $^{35}$S-methionine.[11]

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The purpose of the present study was to determine if radioautography would demonstrate the exclusive incorporation of tryptophan into the granules of the CGT cells and to suggest a route of synthesis for the material within the granules.

## Materials and Methods

Six male rats of 230 to 250 gm each were injected intravenously with 1 μc/gm body weight of D,L-tryptophan-2,3-$^{3}$H* in 0.89% saline. The rats were killed one at a time at intervals beginning at 20 minutes and ending at 180 minutes after injection. The intact, paired SMGs were removed and pieces of each were placed in Bouin's fixative for light microscopy and 5% glutaraldehyde in 0.1 *M* phosphate buffer (pH 7.2) for electron microscopy. Tissues for light microscopy were processed histologically and radioautographed by the method of Rogers,[12] with an emulsion.† Tissues for electron microscopy were dehydrated in an ethanol series and embedded in resin.‡ The radioautographic method used for electron microscopy was adapted from that of Caro and van Tubergen[13] with another emulsion.§ Pieces of organs from rats injected and not injected were taken for controls. The thin sections were examined under an electron microscope‖ after staining with lead citrate.[14]

## Results

Silver grains were observed at all time intervals over the nuclei and cytoplasm of SMG acinar cells, CGT cells, and the cells

* New England Nuclear Corp., Boston, Mass.
† Kodak NTB3, Rochester, NY.
‡ Epon-Araldite, Shell Chemical Co., New York, NY.
§ Ilford L4, Ilford Ltd., Essex, Eng.
‖ Siemans Elmiskop 1 electron microscope, Siemens America Inc., New York, NY.

of the other organs. However, the number of grains over pancreatic acinar cells was five to ten times greater than over the cells of the SMG. The grain counts over cells of the liver and intestine were intermediate. The pattern of labeling over both acinar and CGT cells of the SMG changed with time, as shown in Figure 1. The greatest amount of labeling over acinar cells occurred at the earlier time intervals over the basal thirds of the cells ($57.8 \pm 3\%$ at 30 minutes), and dropped to significantly lower levels ($38.4 \pm 1\%$) by two hours after injection ($P < 0.002$; Table 1). In contrast, labeling over the apical third of the acinar cells was lowest ($13.0 \pm 1.6\%$) at 30 minutes, and rose to significantly higher levels ($42.2 \pm 1.2\%$) by three hours after injection ($P < 0.002$). Similarly, grain counts were done for the CGT. As with acinar cells, labeling over the basal half of the CGT cells was greatest ($73.3 \pm 3\%$) at early time intervals (20 minutes), but gradually dropped to much lower levels ($44.4 \pm 1.6\%$) after two hours ($P < 0.002$; Table 2). Labeling over the apical half of CGT cells was lowest ($27.7 \pm 3\%$) at 20 minutes after injection, but gradually increased to a maximum ($55.6 \pm 1.6\%$) at two hours after injection ($P < 0.002$). The lumina of both acini and CGT were so small that labeling was not evident. However, label was present over lumina of striated ducts and excretory ducts at each time interval.

Electron microscope radioautographs of

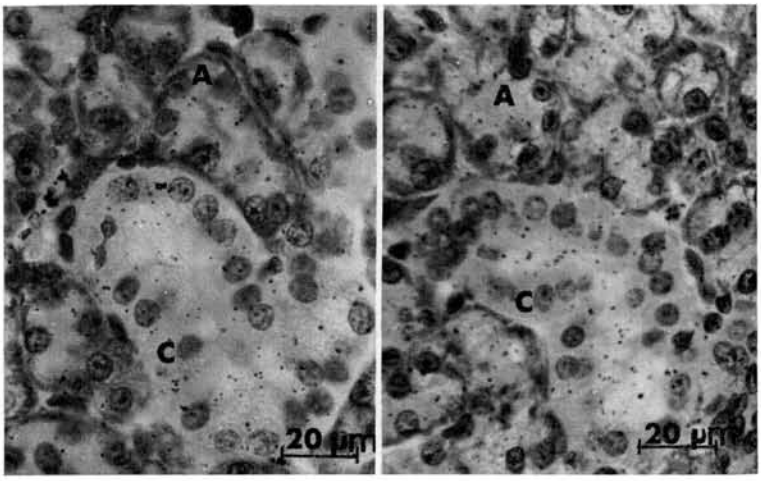


FIG 1.—Rat submandibular gland after intravenous injection of $^3$H-tryptophan. At 30 minutes (*left*) and 180 minutes (*right*), silver grains are observed over the acinar cells (*A*) and convoluted granular tubule (*C*). However, most granules are observed over basal portions of both cell types at 30 minutes and over the apical portions at 180 minutes (H&E stain, orig mag $\times 500$).

TABLE 1

SILVER GRAIN COUNT PER 100 µm$^2$ OVER ACINAL CELLS AT VARIOUS TIME INTERVALS AFTER INTRAVENOUS INJECTION OF $^3$H-TRYPTOPHAN

| Zone | $\bar{X} \pm$ SE* at Time Intervals (%) | | | | | |
|---|---|---|---|---|---|---|
| | 20 min | 30 min | 60 min | 90 min | 120 min | 180 min |
| Basal | 48.5±1.2 | 57.8±3.0 | 47.5±1.5 | 40.0±2.6 | 38.4±1.0 | 42.2±1.4 |
| Middle | 38.0±2.2 | 29.2±1.9 | 23.8±1.7 | 30.6±1.3 | 22.8±2.8 | 15.6±1.3 |
| Apical | 13.5±1.2 | 13.0±1.6 | 28.7±0.8 | 29.4±2.2 | 38.8±2.7 | 42.2±1.2 |
| Total grain count corrected for background | 1.6±0.2 | 2.1±0.1 | 2.0±0.1 | 2.1±0.2 | 2.5±0.3 | 3.1±0.2 |

*Note*: Grain counts were made over between 200 and 500 acinar cells at each time interval.
* SE, standard error.

TABLE 2

SILVER GRAIN COUNT PER 100 μm² OVER CONVOLUTED GRANULAR TUBULES AT VARIOUS TIME INTERVALS AFTER INTRAVENOUS INJECTION OF $^3$H-TRYPTOPHAN

| Zone | $\bar{X} \pm$ SE* at Time Intervals (%) | | | | | |
|---|---|---|---|---|---|---|
| | 20 min | 30 min | 60 min | 90 min | 120 min | 180 min |
| Basal | 73.3±3.0 | 68.4±1.5 | 53.7±2.5 | 52.6±2.0 | 44.4±1.6 | 44.5±2.4 |
| Apical | 27.7±3.0 | 31.6±1.5 | 46.3±2.5 | 47.4±2.0 | 55.6±1.6 | 54.6±2.5 |
| Total grain count corrected for background | 1.3±0.1 | 1.7±0.1 | 2.2±0.2 | 2.4±0.1 | 3.6±0.2 | 3.1±0.2 |

* SE, standard error.

the acinar cell showed over half of the label associated with the rough endoplasmic reticulum (RER) at 30 minutes after injection (Table 3). At the same time, about one quarter of the label was present over the secretory granules. However, the amount of label gradually decreased over the RER to about 28% by three hours after injection, and increased over the secretory granules to about 48% by the same time period. Labeling of the Golgi apparatus was low at all time periods.

Unlike acinar cells, cells of the CGT do not contain a large amount of RER. The base is filled with mitochondria that lie parallel to deep indentations of the plasma membrane, and surround the nucleus (Fig 2, *left*). Interspersed among these organelles are many polyribosomes, a few cisternae of RER, and isolated membranes of the perinuclear Golgi complex. Above the mitochondrial zone are vacuoles and dense granules that resemble serous secretory granules (Fig 2, *right*).

Electron microscope radioautographs of CGT cells showed that most of the label (about 49%) at 30 minutes after injection was associated with the basal cell membrane, the adjacent mitochondria, or both (Table 4). At the same time interval, about a quarter of the label was found free in the cytoplasm, but only about 10% was associated with the apical dense granules or apical cytoplasm. The amount of label decreased over the basal cell membranes and mitochondria to 36.6% by three hours after injection, but at the same time gradually increased to about 26.5% over the apical dense granules and apical cytoplasm by the three hour interval. The amount of label over RER in the CGT was never more than about 10%.

## Discussion

The results of this study indicate that there are similarities in protein synthetic activity between CGT cells and acinar cells of the SMG. Both cell types contain granules in their apical portion into which $^3$H-tryptophan or a product thereof is incorporated. Label that results from intravenous injection of $^3$H-tryptophan appears to move through CGT cells and acinar cells from base to apex and is secreted into the lumina.

The sparseness of RER and the Golgi complex as compared with other exocrine cells,[7,15,16] suggests that the product of the

TABLE 3

ULTRASTRUCTURAL DISTRIBUTION OF SILVER GRAINS OVER ACINAR CELLS AT VARIOUS TIME INTERVALS AFTER INJECTION OF $^3$H-TRYPTOPHAN

| Site | Radioautographic Grains (%) | | | |
|---|---|---|---|---|
| | 30 min | 60 min | 120 min | 180 min |
| Rough endoplasmic reticulum* | 58.7 | 45.8 | 27.6 | 30.2 |
| Nucleus | 7.5 | 0 | 8.7 | 8.5 |
| Golgi complex | 4.2 | 8.1 | 6.5 | 4.6 |
| Mitochondria | 1.6 | 0 | 5.8 | 1.3 |
| Secretory granules | 24.3 | 45.8 | 48.1 | 48.0 |
| Other sites† | 3.3 | 0 | 2.8 | 7.2 |

* Grain counts included silver grains over basal, perinuclear rough ER, and silver grains over rough ER between secretory granules.

† Counts included grains over acinar lumen.

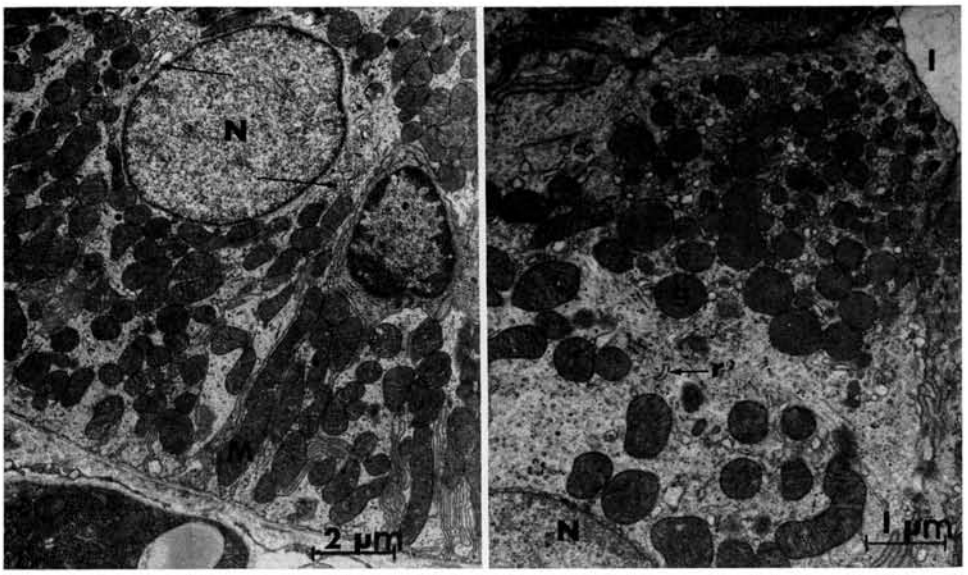


FIG 2.—Basal (*left*) and apical halves (*right*) of convoluted granular tubule cell. *M,* mitochondria, arranged with long axes perpendicular to basal lamina, and parallel to plasma membrane infoldings. *N,* nucleus, is located either in center of cell or basally, depending on number of granules present in cell. Golgi membranes, *arrows,* are sparse, and lie around the nucleus. Strands of rough endoplasmic reticulum (*r*) are usually distributed between nucleus and zone of vacuoles and dense granules (*g*). Lumen (*l*), of duct is apical to granules (uranyl acetate and lead citrate stain; *left,* orig mag ×5,200; *right,* orig mag ×10,800).

secretory granules may be concentrated and stored at a much slower rate. Another possibility is that the exportable protein may not be synthesized exclusively on ribosomes bound to the endoplasmic reticulum, as proposed for exportable pancreatic protein by Siekevitz and Palade.[17] The relatively high grain count over the organelle-free cytoplasm (Table 4) suggests that exportable protein may be transported through the cytoplasm to the region of the vacuoles and secretory granules. This alternate route of transport has been suggested for exportable pancreatic enzymes in several studies.[18] The secretory granules appear to accumulate with eventual discharge in a cyclic fashion, because many CGT cells normally have no granules but others are engorged.[9]

Preliminary data seem to indicate that tryptophan is not incorporated exclusively into CGT cells, but like other amino acids, is incorporated into protein of all cells. Also, it appears that the CGT cells

TABLE 4

ULTRASTRUCTURAL DISTRIBUTION OF SILVER GRAINS OVER CONVOLUTED GRANULAR TUBULE CELLS AT VARIOUS TIME INTERVALS AFTER INJECTION OF $^3$H-TRYPTOPHAN

| Site | Radioautographic Grains (%) | | | |
|---|---|---|---|---|
| | 30 min | 60 min | 120 min | 180 min |
| Basal cell membranes, mitochondria | 49.4 | 37.7 | 39.1 | 36.6 |
| Nucleus | 11.9 | 11.2 | 7.1 | 11.3 |
| Golgi complex | 2.3 | 0.7 | 0.5 | 0.6 |
| Rough endoplasmic reticulum | 3.3 | 9.1 | 6.0 | 10.6 |
| Secretory granules, apical cytoplasm | 9.0 | 21.8 | 22.0 | 26.5 |
| Other sites* | 23.7 | 19.7 | 24.8 | 13.8 |

* Majority of these grains were found over organellefree cytoplasm.

synthesize and secrete granules that contain protein, as do other exocrine cells. Our preliminary observations of the mechanism of protein synthesis by CGT cells and the route of the label through the cells showed only that it progresses from the base to the apex. The early association of label with mitochondria was probably because of the metabolism of tryptophan to alanine and 3-hydroxykynurenine in the mitochondria.[19] However, the resulting alanine and the unmetabolized $^{3}$H-tryptophan may still be available for incorporation into protein. The existence of a system for synthesizing and secreting proteins by CGT cells would require an expansion of our morphologic definition of a secretory cell and would offer another interesting model for the study of exocrine cells.

## Conclusions

The number of silver grains over the secretory cells of rat submandibular glands after injection of tritiated tryptophan indicates that the label was incorporated into both acinar cells and convoluted granular tubule cells. This data added further evidence that both kinds of cells are exocrine in nature and synthesize and secrete products that have a protein component.

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