

Autoradiographic Observations on the Silk Glands of *Bombix mori*. BY M. RABINOVITCH AND I. VUGMAN.* (From the Laboratory for Cell Physiology, Department of Histology and Embryology, Faculty of Medicine, University of São Paulo, Brazil.)‡

Brachet (1) first called attention to the correlation between tissue RNA content and protein synthesis and to the RNA richness of the silk glands of *B. mori*. This high RNA content (2), cytological characteristics (3), and synthesis of a specific protein-fibroin—with peculiar properties and amino acid composition (4)—suggested an autoradiographic study of the incorporation of radioactive amino acids into the silk glands.

Fibroin is synthesized by the posterior part of the silk glands (5). The tubular silk glands are lined by large cells, filled with ergastoplasm erroneously interpreted as secretion by Lesperon (3). In their apices are found hyaline globular masses and complex structures constituted by a filamentous grid, stainable by phosphotungstic acid hematoxylin. This apical structure is presumably related to the secretion of fibroin into the lumen of the gland. Cell nuclei are branched and contain numerous nucleoli.

Materials and Methods

Two series of experiments were made. In the first, silkworms of the 5th instar, weighing 1.60 to 2.00 gm. and 3.50 to 4.50 gm., were injected in the body cavity respectively with 0.05 ml. = 0.5 μ c. and 0.1 ml. = 1 μ c. of glycine-1- C^{14} dissolved in 0.85 per cent NaCl. Groups of silkworms, each composed of 6 worms of each weight, were killed at 1, 2, 6, 12, and 24 hours after the injection. Silk glands were fixed either in 10 per cent formaldehyde, buffered with phosphate to pH 7.1, or Carnoy. In a second series of experiments, silkworms in the 5th instar, weighing 3 to 4 gm. received 0.03 ml. = 3 μ c. of C^{14} -glycine, and the glands were collected 15, and 30 minutes, and 1, 2, 6, 12, and 24 hours later, and fixed in 10 per cent formaldehyde. Four larvae were studied for each time period. All glands were paraffin-embedded and cut at 3 μ . Slides were deparaffinized, washed for a few hours in tap water, and following that by several changes of a M/100 *d*-l-glycine solution and distilled water. Some slides were treated with 0.02 per cent crystalline pancreatic ribonuclease. Slides were covered with Kodak Ltd. stripping emulsion and developed after a month's exposure (6). The unstained sections were examined by phase contrast microscopy after mounting in water.

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RESULTS

The two fixatives used gave the same autoradiographic results, but tissue preservation was better after formaldehyde fixation. As the two experimental series gave similar results, they are jointly described. No clear influence of the larvae weight was found. Ribonuclease treatment of the material did not conspicuously change the appearance of the autoradiographs (ARGs). Over the nuclei the ARG was negative. At 15 (Figs. 1 and 2) and 30 minutes after the injection of the isotope the ARG showed strong activity in the cell body. In the 1 hour group (Figs. 3 and 4) cellular activity was weaker, but very strong over the cell apex, and there was some over the periphery of the material in the lumen. At 2 hours (Figs. 5 and 6) the ARG was similar to that of the previous group, except in showing more intense activity in the periphery of the material excreted in the lumen. At 12 (Fig. 7) and 24 hours the ARG was faint over the cell body and cell apex; it was strong over the material secreted into the glandular lumen, frequently being limited to its central part.

DISCUSSION

Radioactive glycine and alanine (7) as well as phenylalanine (8, 9) injected into the body of silkworms are incorporated in the fibroin of cocoons. Incorporation of administered C^{14} -glycine into proteins and nucleic acids of silk gland cell fractions (10), as well as *in vitro* synthesis of fibroin in synthetic media by silk glands (11), have also been reported.

Our results show that 15 minutes after its administration, C^{14} -glycine in an insoluble form is found all over the cell body of the posterior silk gland cells. Although the possibility of unspecific adsorption cannot be entirely ruled out, this rapid trapping of the amino acid agrees with data (12) obtained with chicken liver slices. At 1 hour a positive ARG was found over the periphery of the secreted material in the gland lumen, suggesting that this may be the time period involved in the synthesis and secretion of fibroin molecules. This result agrees with those of Shimura *et al.* (10) obtained with a trichloroacetic acid preparation of fibroin and silk gland proteins, and with those of Junqueira *et al.* (13) obtained with rat pancreas.

After 6, 12, and 24 hours the ARG over the cell body became progressively weaker, while it moved towards the center of the material contained in the lumen. In preliminary results with C¹⁴-adenine, no ARGs were obtained, while after S³⁵-methionine administration the ARG was diffuse over the cell body. The results of the ribonuclease treatment suggest that the incorporation of C¹⁴-glycine into RNA, if any, was much smaller than that into protein; this agrees with Takeyama *et al.* (11) who could not find any *in vitro* incorporation of C¹⁴-glycine into purine bases of RNA.

SUMMARY

Glycine-1-C¹⁴ was administered to *B. mori* larvae and its incorporation in the silk glands followed autoradiographically. After 15 and 30 minutes the ARGs showed strong and diffuse activity in the cell cytoplasm. At 1 hour it was mainly found over the cell apex and newly formed secreted material. At 12 and 24 hours the activity was localized mainly in the material secreted into the lumen.

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EXPLANATION OF PLATE 154

- S, intraluminal secretion.
 A, cell apex.
 N, nucleus.
 C, cytoplasm.

FIGS. 1 to 7. Autoradiographs of *B. mori* posterior silk gland sections at different times after glycine-1-C¹⁴ (3 μ c.) injection.

FIG. 1. Gland fixed 15 minutes after isotope administration. Phase contrast. Note intraluminal secretion (S), cell apex (A), nucleus (N), and "basal" cytoplasm (C).

FIG. 2. Brightfield photograph of same field, focus on emulsion. Absence of ARG over nuclei (N), cell apex (A), and secretion (S); diffuse ARG over cytoplasm (C).

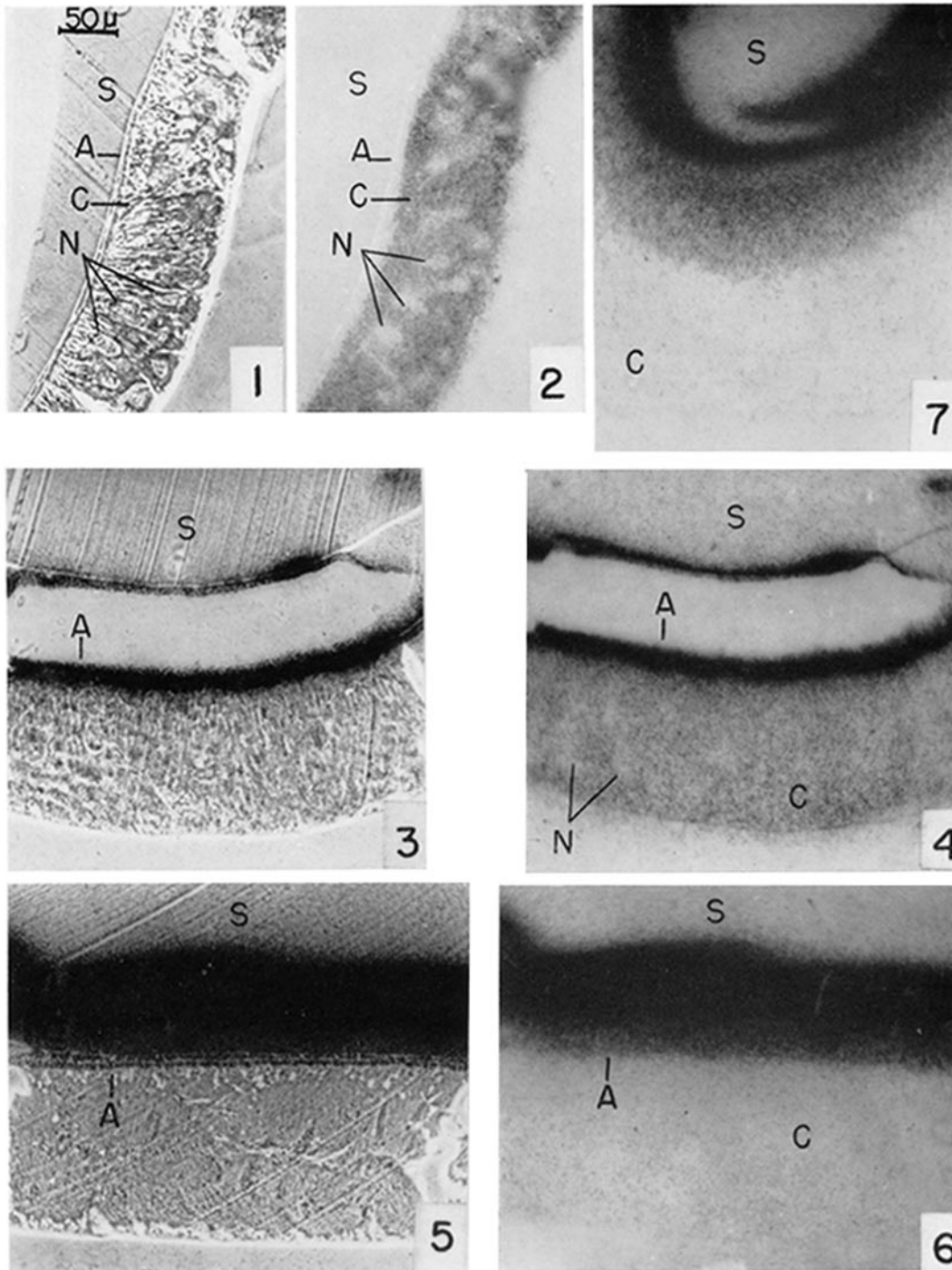
FIG. 3. Gland fixed 1 hour after isotope administration, phase contrast. To be compared with Fig. 4. Outlines of nuclei are indistinct.

FIG. 4. Brightfield photograph of same field, focus on emulsion. Strong ARG over cell apex (A) and peripheral part of intraluminal secretion (S) artifactually separated from A. Diffuse ARG over cytoplasm, clear areas due to nuclei.

FIG. 5. Gland fixed 2 hours after isotope administration. Phase contrast. To be compared with Fig. 6.

FIG. 6. Same field of Fig. 5, brightfield photograph, focus on emulsion. Strong ARG over peripheral part of secretion (S); weaker ARG over cell apex (A), and cytoplasm (C).

FIG. 7. Brightfield photograph, 12 hours after isotope administration. Strong ARG over intraluminal secretion (S), weak ARG over cytoplasm (C).



(Rabinovitch and Vugman: Silk glands of *B. mori*)