A NOTE ON AUTORADIOGRAPHY; AN INTRODUCTION OF VITAMIN A-STORING CELLS^{1,2}

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Autoradiography (ARG) has revealed many functions of cells and their organelles and this has made it one of the most useful techniques in modern biology. The technical standard has been improved and maintained by many investigators (13, 15, 21). The aim of this note is not to discuss the technical aspects of ARG, but to make a few brief comments on some of the works which have contributed to the advance of biological knowledge.

Many of the classic studies in electron microscope autoradiography (EMARG) have been performed with precursors of nucleic acids, proteins and carbohydrates. As shown in Table 1, these precursors are still the most popular ones. Since ARG silver grains are presumed to indicate the site of radioactive precursors which have been incorporated and survived preparation procedures, lipid and lipid soluble materials are not adequate as precursors. Many attempts have been made to keep these materials at the sites where they are incorporated, because their metabolism is one of the most important matters in cell biology (for review, see 19). To locate silver grains on cells and organelles, the resolution of ARG must be considered. The resolution of EMARG is much less than that of EM itself. Therefore, it is not adequate to discuss the localization of silver grains on structures which have less dimension than ARG resolution. EMARG resolution is influenced by such factors as energy of beta-particles, thickness of sections, thickness and quality of nuclear track emulsions and photographic procedures. It is usually estimated as 1000–2000 Å (22, 23). A more sophisticated technique has been reported to make the resolution less than 400 Å (14), but it can not be achieved through the use of ordinary procedures.

ARG applications to biological materials have been achieved for the detection of the intracellular movements of macromolecules in association with cellular structures and for cytological labeling with specific markers. ARG works of this type are as follows;

A) The intracellular movement of macromolecules:

This movement corresponds with the synthesis, transport and secretion of macromolecules. The following steps can be traced in the formation of zymogen granules; proteins are synthesized in rough endoplasmic reticulum and condensed in the Golgi apparatus, then matured for secretion (11). Carbohydrates moieties are added to proteins in the Golgi apparatus (17). Metabolism of membrane

1) Original articles have been published by authors, K. Hiroawa and E. Yamada (Department of Anatomy, Faculty of Medicine, University of Tokyo).

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TABLE 1. *Papers by Autoradiography from the J. Cell Biology, 57 (1973)–70 (1976)*

	Total	EM	LM
Precursors of nucleic acids	46	11	35
Precursors of amino acids	23	9	14
Lipids and lipid solubles	8	6	2
Carbohydrates	7	4	3
Amines	1	1	0
¹²⁵ I			
α -bungarotoxin	2	2	0
Fab-	1	1	0
Gonadotropin	1	0	1
Ouabain	1	0	1
Carrier free	2	1	1
⁵⁵ Fe	1	1	0
³⁵ S	1	1	0
³³ P	1	(ARG of chromatography)	
⁴⁵ Ca	1	0	1
¹³ N	1	0	1
	97	37	59

components is difficult to detect with this technique. However, one exception is the formation and migration of retinal rod disk membranes which are a pile of homogeneous membranes; labeled rhodopsin molecules are revealed to stay in the membranes and old disk membranes migrate towards the retinal pigment epithelium into which they are shedded off (26, 27). Axonal flow in nerve cells has also been analysed with the use of ARG (2). What is required in order to analyse these movements is not the high resolution but the localization of silver grains on cellular organelles.

B) Specific markers:

One of the most popular and useful markers is ³H-thymidine, as is shown in Table 1. This has been used to mark differentiated (postmitotic) cells at LMARG. (4), but EM autoradiographs are prepared when structural analysis becomes necessary to determine the nature of labeled cells (16). Iodine (¹²⁵I) can also be utilized in various ways (see Table 1), but in particular is an important marker for thyroid glands, in which it is heavily concentrated. Primordial thyroid cells were identified in the ammocoetes by the use of this method (3). The author has conducted work on ³H-vitamin A in order to examine the distribution of vitamin A-storing cells in mice (8). Vitamin A-storing cells are also one example of how ARG works when identification of any special cell types is required. Though the vitamin is not completely preserved through the preparation procedures for EM, amounts of lipid droplets survive the procedures (9) and serve as markers of vitamin A-storing cells.

Vitamin A-storing cells in the mouse:

Radioactive vitamin A was found highly concentrated in lipid droplets of

FIG. 1. A fat-storing cell in the mouse liver. Lipid droplets are heavily labeled. Note dilated cisternae of rough endoplasmic reticulum and nuclear envelope (arrow). Mag. $\times 11,000$

FIG. 2. Lipid droplets in hepatocytes are found labeled with use of water-miscible resin embedding method. These droplets are not radioactive with routine methods. Mag. $\times 10,000$ (from Fig. 10, Ref. 5)

FIG. 3. A labeled cell in the tunica propria mucosa of small intestine. Thick collagen fibrils surround the cell. Rough endoplasmic reticulum (rER). Mag. $\times 18,000$

FIG. 4. A section of lung septal cells without ARG. One can not imagine that these lipid droplets contain vitamin A from this micrograph. Capillaries (ca). Mag. $\times 22,000$

FIG. 5. A cross section of zona fasciculata in the adrenal gland. It is easily recognized that labeled cells are only in the space between the sinusoid endothelium and parenchymal cells. Mag. $\times 3,000$

fat-storing cells (12, 24) in the liver, whereas it was found that radioactivity in lipid droplets of hepatocytes did not survive routine preparations (5). In order to label experimental animals, two mice (male and female) were kept on a diet containing vitamin A-11,12- $^3\text{H}_2$ acetate (7.7 mCi in 22.4 mg) for 1 week. Organs of interest were immersed in a fixative (1% glutaraldehyde and 1% formaldehyde in 0.1 M cacodylate buffer). Small pieces of organs were postfixated with 1% OsO_4 in the same buffer and embedded in Araldite mixture. Half micron sections were processed for LMARG (nuclear track emulsion; Sakura NR-M2) and thin sections for EMARG (Ilford L4). Portions of liver were embedded in water-miscible resin (18) and sectioned for EMARG.

Heavily labeled cells were found in the liver, lung, digestive tract (esophagus to large intestine), adrenal gland, spleen, ductus deferens, and uterus. EMARG showed the common morphological features of these labeled cells; heavily labeled lipid droplets, dilated cisternae of rough endoplasmic reticulum, many free ribosomes, attenuated cellular processes, filaments assembled beneath the plasma membrane, and a close relationship to capillaries. These are very similar to those of fibroblasts but fibroblasts in the dermis were not labeled and lipid droplets were absent. In the liver (5), heavily labeled cells (fat-storing cells) were located in the Disse's space extending their processes onto the surface of hepatocytes (Fig. 1). Water-miscible resin embedded specimens have many electron dense lipid droplets in fat-storing cells as well as in hepatocytes. Besides the lipid droplets in fat-storing cells, lipid droplets in hepatocytes were found labeled (Fig. 2). This suggests that

vitamin A in hepatocytes is more labile to dehydration procedure than that in fat-storing cells. The chemical forms of vitamin A in the two kinds of droplets would be different each other. Only vitamin A in fat-storing cells is firmly bound to other components for storage. In the following organs, only the storage form will be considered. In the digestive tract, labeled cells were found in the tunica propria mucosa as well as in the submucous layer. Their population was highest in the small intestine (10). These cellular processes extended beneath the basal lamina of epithelium or around capillaries. Collagen fibrils were found to be thick around the cells (Fig. 3). In the lung, the cells were located in the septum of alveoli in a close relationship with both the alveolar cells and capillaries (6). Although they had no basal lamina, they were in close contact with elastic and collagen fibrils (Fig. 4). Labeled cells were found in the adrenal gland between unlabeled parenchymal cells and sinusoid. They had a morphology similar to that of fat-storing cells in the liver (7). Lipid droplets in cells of the zonula fasciculata were not found labeled with the use of ordinary EMARG (Fig. 5). Cells in other organs are now being investigated in our laboratory. The presence of vitamin A-storing cells in many organs strongly suggests that a vitamin A-storing cell system exists in the mouse (25). Their morphological resemblances to fibroblasts and geographical relationship to collagen fibrils imply that these cells function to synthesize collagen fibrils as well as to store vitamin A. Accumulation of vitamin A in the liver has been reported to stimulate collagen synthesis in human and rats (1, 20), and to also increase the number and size of lipid droplets in fat-storing cells (12, 40). The evidence suggests that if there is any collagen synthesis in vitamin A-storing cells it is regulated by different processes from that in skin.

In summary, the identification of cells which had vitamin A-storing activity was successful only with ARG. However, other cytological and biochemical methods must be applied in order to establish the concept of a vitamin A-storing cell system.

REFERENCES

1. Bahu, R., Minick, O. T., Inouye, T., Kent, G. and Popper, H.: The dual function of hepatic lipocytes. *J. Cell Biol.* 67; 15a, 1975.
2. Droz, B.: Protein metabolism in nerve cells. *Int. Rev. Cytol.* 25; 363-390, 1969.
3. Fujita, H. and Honma, Y.: Iodine metabolism of the endostyle of larval lampreys, ammocoetes of *Lampetra japonica* Electron microscopic autoradiography of ^{125}I . *Z. Zellforsch.* 98; 525-537, 1969.
4. Fujita, S.: Matrix cell and cytogenesis in the central nervous system. *J. Comp. Neurol.* 120; 37-42, 1963.
5. Hirosawa, K. and Yamada, E.: The localization of the vitamin A in the mouse liver as revealed by electron microscopic radioautography. *J. Electron Micros.* (Tokyo) 22; 337-346, 1973.
6. Hirosawa, K. and Yamada, E.: Localization of ^3H -vitamin A in the mouse lung as revealed by electron microscope radioautography. *Acta Anat. Nippon* 49; 60, 1974.
7. Hirosawa, K. and Yamada, E.: Vitamin A-storing cells; an electron microscope autoradiographic study. *Proc. 10th Int. Cong. Anat.*, 1975, p. 482.
8. Hirosawa, K. and Yamada, E.: Vitamin A storing cells in the mouse. *J. Cell Biol.* 70; 269a, 1976.
9. Hirosawa, K. and Yamada, E.: Localization of vitamin A in the mouse retina as revealed by radioautography. *Jap. J. Ophthalmol.* Struct. Eye III, ed. by E. Yamada and S. Mishima

- 1976, pp.165-175.
10. Hirosawa, K. and Yamada, E.: Localization of vitamin A in the small intestine of mouse; an electron microscope radioautographic study. *Cell Tissue Res.* 177; 57-65, 1977.
 11. Jamieson, J. D. and Palade, G. E.: Intracellular transport of secretory proteins in the pancreatic exocrine cell II. Transport to condensing vacuoles and zymogen granules. *J. Cell Biol.* 34; 579-615, 1967.
 12. Kobayashi, K. and Takahashi, Y.: Effect of the administration of large dose of vitamin A on the fine structure of rat liver with special reference to changes in fat-storing cell. *Arch. Histol. Jap.* 33; 421-443, 1971.
 13. Kopriwa, B. M.: A reliable, standardized method for ultrastructural electron microscopic radioautography. *Histochemie* 37; 1-17, 1973.
 14. Mizuhira, V. and Futaesaku, Y.: The electron microscope autoradiography. Limit of the high resolution and diffusable substances. *Radioisotopes* 24, 573-587, 1975.
 15. Mizuhira, V. and Futaesaku, Y.: Limits of resolution in electron microscope autoradiography. In *Recent Progress in Electron Microscopy of Cells and Tissues*, ed. by E. Yamada, V. Mizuhira, K. Kurosumi and T. Nagano, Igaku Shoin, Tokyo, 1976, pp. 147-174.
 16. Mori, S. and Leblong, C. P.: Electron microscopic identification of three classes of oligodendrocytes and a preliminary study of their proliferative activity in the corpus callosum of young rats. *J. Comp. Neurol.* 139; 1-30, 1970.
 17. Neutra, M. and Leblond, C. P.: Synthesis of the carbohydrate of mucus in the Golgi complex as shown by electron microscope radioautography of goblet cells from rats injected with glucose- H^3 . *J. Cell Biol.* 30; 119-136, 1966.
 18. Pease, D. C.: Glutaraldehyde-urea, copolymerized with glycol methacrylate by ultraviolet. Proc. 30th Ann. Meeting EMSA, 1972, pp. 142-143.
 19. Roth, L. J. and Stumpf, W. E.: *Autoradiography of diffusable substances*. Acad. Press, New York and London, 1969.
 20. Russell, R. M., Boyer, L. J., Bagheri, S. A. and Hruban, Z.: Hepatic injury from chronic hypervitaminosis A resulting in portal hypertension and ascites. *New Engl. J. Med.* 291; 435-442, 1974.
 21. Salpeter, M. M. and Bachmann, L.: Autoradiography with the electron microscope. A procedure for improving resolution, sensitivity, and contrast. *J. Cell Biol.* 22; 469-477, 1964.
 22. Salpeter, M. M. and Salpeter, E. E.: Resolution in electron microscope radioautography. *J. Cell Biol.* 41; 1-20, 1969.
 23. Salpeter, M. M. and Salpeter, E. E.: Resolution in electron microscope radioautography II. Carbon 14. *J. Cell Biol.* 50; 324-332, 1971.
 24. Wake, K.: "Sternzellen" in the liver: Perisinusoidal cells with special reference to storage of vitamin A. *Am. J. Anat.* 132; 429-462, 1971.
 25. Yamada, E. and Hirosawa, K.: The possible existence of a vitamin A-storing cell system. *Cell Struct. Funct.* 1; 201-204, 1976.
 26. Young, R. W. and Dorz, B.: The renewal of protein in retinal rods and cones. *J. Cell Biol.* 39; 169-184, 1968.
 27. Young, R. W. and Bok, D.: Participation of the retinal pigment epithelium in the rod outer segment renewal process. *J. Cell Biol.* 42; 392-403, 1969.