A NOTE ON AUTORADIOGRAPHY: AN INTRODUCTION OF VITAMIN A-STORING CELLS

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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. Hirosawa and E. Yamada (Department of Anatomy, Faculty of Medicine, University of Tokyo).
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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

<table>
<thead>
<tr>
<th>Precursors of nucleic acids</th>
<th>Total</th>
<th>46</th>
<th>11</th>
<th>35</th>
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<tr>
<td>Precursors of amino acids</td>
<td></td>
<td>23</td>
<td>9</td>
<td>14</td>
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<tr>
<td>Lipids and lipid solubles</td>
<td></td>
<td>8</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td></td>
<td>7</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Amines</td>
<td></td>
<td>1</td>
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<td>0</td>
</tr>
<tr>
<td>125I</td>
<td></td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>a-bungarotoxin</td>
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<td>1</td>
<td>1</td>
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</tr>
<tr>
<td>Fab</td>
<td></td>
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<td>1</td>
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<tr>
<td>Gonadotropin</td>
<td></td>
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<td>1</td>
</tr>
<tr>
<td>Ouabain</td>
<td></td>
<td>1</td>
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<td>1</td>
</tr>
<tr>
<td>Carrier free</td>
<td></td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>55Fe</td>
<td></td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>35S</td>
<td></td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>33P</td>
<td></td>
<td>1</td>
<td>(ARG of chromatography)</td>
<td></td>
</tr>
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<td>45Ca</td>
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<td>1</td>
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<td>13N</td>
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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. ×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. ×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. × 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. × 22,000
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 con-
taining vitamin A-11,12-3H2 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 postfixed with 1% OsO4
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 ribo-
somes, 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 paren-
chymal 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 colla-
genfibris 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.

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