Topic 1: Autoradiography

**AN ATTEMPT OF QUANTITATIVE ANALYSIS FOR ELECTRON
MICROSCOPE AUTORADIOGRAPHY**

VINCI MIZUHIRA and YUTAKA FUTAESAKU

Tokyo Medical and Dental University, Tokyo 113

We studied the biosynthesis of testosterone in the rat testis, by means of electron microscope autoradiography. The male rats were divided into two groups. The first group was X-ray irradiated with 1,000 Roentgen units, centered to the testis, and gonadotropic hormone was injected daily for two weeks. The other group was used as control. Tritiated pregnenolone was injected to the experimental groups. 30 min after the injection, the rats were autopsied. The testes were fixed with ordinary method, or with saturated digitonine added to the fixative, and dehydration media. After, embedding in Epon, the tissue was sectioned and prepared for electron microscope autoradiography as described previously.^{1,2,3)}

Thin sections were mounted on a grid, evaporated with carbon, covered with Sakura NR-H2 nuclear research emulsion, then exposed for four weeks in a refrigerated darkbox. After the exposure, the grid was developed with Elon ascorbic acid-developer at 17°C for 14 min, immediately after brief treatment (10 sec.) with gold chloride solution.¹⁻⁴⁾

Developed silver grains were observed to be scattered on smooth membranes and mitochondria. In the enlarged pictures, developed silver grains were observed on smooth membranes, mitochondria and lipid droplets.

In addition, testes were homogenized and divided by centrifugation into fractions, such as nuclear, mitochondrial, microsomal and supernatant with usual manner. These fractions were examined with thin layer chromatography, and liquid scintillation spectrometry. One half of the fractions were fixed with ordinary, the other half with the digitonizing method as mentioned above, then embedded in Epon, and prepared for electron microscope autoradiography.

On the electron microscope autoradiograms of the unpurified microsomal fraction from the tritiated pregnenolone injected rat testis, developed silver grains were only located on the smooth membranes, but were never observed on the ribosomal elements.³⁾

In the case of the purified fraction, we observed developed silver grains scattered on the microsomal membranes, as shown in Fig. 4.

The biosynthesis of a hormone, in this case testosterone, can be studied by biochemical cell fraction, but also by autoradiography.

The biochemical approach destroys the tissue, while in the histochemical procedures tissue structures are left relatively intact.

The biochemist's testicular microsomal fractions are a mixture of testicular elements, that is, seminiferous tubules, interstitial, that is, Leydig cells as well as

other the testis was irradiated with high units of Roentgen and treated with gonadotropic hormone. From the biochemical data, it is assumed that testosterone is synthesized at the smooth membranes of the Leydig cells.

However, nobody can identify which microsomes come from Leydig cells, and which microsomes come from the other testicular components, if one looks at electron micrograms of the testicular fractions.

Since we can not identify the origin of the developed silver grains in the microsomal fraction, we found it necessary to establish a cell-volume-ratio between the cells of the seminiferous tubules and the Leydig cells. After this, we related this ratio of cell-volume to the radioactivity associated with the microsomes, in order to obtain values pertaining to the seminiferous tubules or Leydig cells respectively. It can be assumed, even if the developed silver grain-count is the same in the Sertoli and Leydig cell, their volume ratio is not always the same.

Therefore, it is necessary to compare the ratio of cell volume per radioactivity or grain-count with the silver-grain-ratios obtained from the different kinds of cell in intact preparations.

We calculated the total cell volume, in addition to the cytoplasmic and mitochondrial volumes, in the following manner.

Testicular tubules, embedded in Epon, were cut the same thickness, photographed and enlarged in the same magnifications. The cross-sectioned seminiferous tubules were cut out from the photogram (Fig. 1), then the width of the tubules measured, weighed, and calculated to the tubular volume as a cylinder.

The volume ratio of the testicular tubules to the interstitial cells was calculated according to the formulas seen in Table 1.

For the calculation of the whole cell-volume of Sertoli cells and interstitial cells these structures were cut from the electron microgram (Fig. 2) in the same way as it was done with light micrograms. After the measuring and weighing of a whole

TABLE 1

Hypothesis 1. We regard seminiferous tubules as columns.	
Hypothesis 2. We regard interstitial cells as beads.	
A:	$V_{\text{sem}} = \pi r_{\text{sem}}^2 h - \pi r_{\text{iss}}^2 h = \pi h (r_{\text{sem}}^2 - r_{\text{iss}}^2)$
	$V_{\text{int}} = \frac{4}{3} \pi r_{\text{int}}^3 \frac{h}{r_{\text{int}}} = \frac{4}{3} \pi r_{\text{int}}^2 h$
*	$V_{\frac{s}{i}} = \frac{V_{\text{sem}}}{V_{\text{int}}} = \frac{3(r_{\text{sem}}^2 - r_{\text{iss}}^2)N_{\text{sem}}}{4r_{\text{int}}^2 N_{\text{int}}} \dots\dots\dots (1)$
	$S_{\text{sem}} = \pi (r_{\text{sem}}^2 - r_{\text{iss}}^2) N_{\text{sem}}, \quad S_{\text{int}} = \pi r_{\text{int}}^2 N_{\text{int}}$
*	$S_{\frac{s}{i}} = \frac{S_{\text{sem}}}{S_{\text{int}}} = \frac{(r_{\text{sem}}^2 - r_{\text{iss}}^2) N_{\text{sem}}}{r_{\text{int}}^2 N_{\text{int}}} \dots\dots\dots (2)$
***	$V_{\frac{s}{i}} = \frac{3}{4} S_{\frac{s}{i}}$

V; volume, S; square measure, N; number of cutting face, r; half diameter
h; thickness of section, sem; seminiferous tubule, int; interstitial cell
iss; interspace of seminiferous tubule

TABLE 2

Hypothesis 1. We regard a section of cell and nucleus as a column.

Hypothesis 2. We regard mitochondria as a globe.

A: volume ratio of cell to nucleus

$$V_{\text{cel}} = \pi r_{\text{cel}}^2 h N_{\text{cel}}, \quad V_{\text{nuc}} = \pi r_{\text{nuc}}^2 h N_{\text{nuc}}$$

$$* \quad V_{\frac{n}{c}} = \frac{r_{\text{nuc}}^2 N_{\text{nuc}}}{r_{\text{cel}}^2 N_{\text{cel}}} \dots\dots\dots (1)$$

$$S_{\text{cel}} = 2\pi r_{\text{cel}} N_{\text{cel}}, \quad S_{\text{nuc}} = 2\pi r_{\text{nuc}} N_{\text{nuc}}$$

$$* \quad S_{\frac{n}{c}} = \frac{r_{\text{nuc}}^2 N_{\text{nuc}}}{r_{\text{cel}}^2 N_{\text{cel}}} \dots\dots\dots (2)$$

$$** \quad \underline{\underline{V_{\frac{n}{c}} = S_{\frac{n}{c}}}}$$

B: volume ratio of cell to mitochondria

$$** \quad V_{\frac{m}{c}} = \frac{\frac{4}{3} \pi r_{\text{mit}}^3 \frac{h}{r_{\text{mit}}} N_{\text{mit}}}{\pi r_{\text{cel}}^2 h N_{\text{cel}}} = \frac{4 \pi r_{\text{mit}}^2 N_{\text{mit}}}{3 \pi r_{\text{cel}}^2 N_{\text{cel}}} = \frac{4}{3} S_{\frac{m}{c}}$$

C: volume ratio of cell to cytoplasm

$$** \quad V_{\frac{p}{c}} = \frac{V_{\text{cel}} - V_{\text{nuc}} - V_{\text{mit}}}{V_{\text{cel}}} = 1 - V_{\frac{n}{c}} - V_{\frac{m}{c}}$$

cell; cell, nuc; nucleus, mit; mitochondria, pls; cytoplasm

cell, the nuclei and mitochondria were cut out from the microgram and also weighed. The nucleus was calculated as a cylinder, and the mitochondrion was calculated as a sphere, as shown in the Tables 2 and 3.

From these calculations, the volume-ratio of interstitial cell to testicular tubule was calculated to be 1 to 38.6, that is, about 1 to 40.

Table 5 shows that the grain count-ratio to $1 \mu^3$ of tissues are; in the Leydig cell: 1175: $1 \mu^3$, and in the seminiferous tubule: 7.9: $1 \mu^3$,

From these calculation, the ratio of radioactivity per volume between the interstitial Leydig cell and seminiferous tubule is calculated to be:

$$\frac{1}{38.6} \times \frac{1175}{7.9} = 3.85$$

that is, the ratio is about 4 : 1.

This result means that if we have five grain-counts in the autoradiogram of the testicular microsomal fraction, four of the developed silver grains originate from Leydig cells, and one is of seminiferous Sertoli cell origin.

In summary, we have used two preparation approaches for the attribution of radioactivity to microsomes in two types of testicular tissues, 30 min after the injection of the testosterone precursor ^3H -pregnenolone.

In autoradiograms prepared from the biochemist's microsomal fraction, as well as prepared from intact testes radioactivity has been demonstrated to localize in microsomal membranes using digitonine saturated fixation and dehydration media for the retention of the 3β -OH steroids.

TABLE 3

Hypothesis 1. We regard cell, nucleus and mitochondria as a globe.					
A: volume of cellorganella					
* $V_{cel} = \frac{4}{3} \pi r_{cel}^3$					
* $V_{nuc} = \frac{4}{3} \pi r_{nuc}^3$					
volume ratio of mitochondria from modelling II is					
$\frac{V_{mit}}{V_{cel}-V_{nuc}} = \frac{\frac{4}{3} \pi r_{mit}^2 h N_{mit}}{\pi r_{cel}^2 h N_{cel} - \pi r_{nuc}^2 h N_{nuc}} = \frac{4}{3} \frac{r_{mit}^2 N_{mit}}{r_{cel} N_{cel}^2 - r_{nuc} N_{nuc}^2}$					
* $V_{mit} = \frac{4}{3} \frac{r_{mit}^2 N_{mit}}{r_{cel}^2 N_{cel} - r_{nuc}^2 N_{nuc}} \frac{4}{3} \pi (r_{cel}^3 - r_{nuc}^3)$					
* $V_{pls} = V_{cel} - V_{nuc} - V_{mit}$					
B: volume ratio of cellorganella					
* $V_{\frac{n}{c}} = \frac{r_{nuc}^3}{r_{cel}^3}$					
* $V_{\frac{m}{c}} = \frac{\frac{4}{3} \frac{r_{mit}^2 N_{mit}}{r_{cel}^2 N_{cel} - r_{nuc}^2 N_{nuc}} \frac{4}{3} \pi (r_{cel}^3 - r_{nuc}^3)}{\frac{4}{3} \pi r_{cel}^3}$					
$= \frac{4}{3} \frac{r_{mit}^2 N_{mit}}{r_{cel}^2 N_{cel} - r_{nuc}^2 N_{nuc}} \frac{r_{cel}^3 - r_{nuc}^3}{r_{cel}^3}$					
* $V_{\frac{p}{c}} = 1 - V_{\frac{n}{c}} - V_{\frac{m}{c}}$					

TABLE 4 Calculated volume of single cell and its organelas

tissues	whole	C. plasm	nuclei	mitochondria	
Interstitial	34.5	19.8	11.0	2.3	u ³
cells	100.0	57.4	31.9	6.7	%
Sertoli	330.0	183.4	130.0	16.6	u ³
cells	100.0	55.6	39.4	5.0	%
Germinal	19.3	8.4	8.3	2.5	u ³
cells	100.0	43.4	42.9	13.2	%

TABLE 5 Grain number ratio / cell and its organel-volumes

Interstitial	14700	9158	2029	3513	grain number
cells	426	463	184	1527	g.n./u ³
Seminiferous	100	50	29	21	g.n.
tubules	0.3	0.3	0.2	1.3	g.n./u ³
Volium ratio of Int.C. / Ser.T.; 1: 38.6					
Grain number ratio to u ³ of tissues Int.C.; 1175 / u ³					
Sem.T.; 7.9 / u ³					
Radioactivity-ratio of Int.C. / Sem.T. ; 4 : 1					

These autoradiograms were used for quantitative evaluation and a comparison of the microsomal fraction with intact histological preparations.

Since, it calculation of the volume-ratio between Leydig cells and Sertoli cells in seminiferous tubules after X-ray irradiation was made, giving a ratio of 1 to 4.

Accordingly most of the radioactivity would be derived from seminiferous tubules if the volume ratio would reflect the microsome ratio between these cells.

However, the autoradiograms from intact testes give different results. In the intact tissue, silver grain-counts were made over cytoplasm excluding nucleus and mitochondria. A comparison between Leydig cells and Sertoli cells, excluding in counts per/ μ^3 , resulted in a ratio of 1,500 to 1 (Table 5).

These results show that the Leydig cell cytoplasm that is, essentially the microsomes, contains most of the radioactivity. This suggests that ^3H -pregnenolone is utilised in these structures, probably for the synthesis of testosterone.

The volume ratio does not appear suitable for the quantitative evaluation of biochemical cell fractions.

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FIG. 1. Light microgram of the rat testis. After the X-ray irradiation and gonadotropine treated, spermatogenic cells and sperm are not seen but the Sertoli cells remained in the testicular tubule. Interstitial cells are seen in the intertubular spaces with blood capillaries.

This photo graph is used for the calculation of the tubular or interstitial cell volume. $400\times$

FIG. 2. Low magnified electron microgram of the interstitial cells. This picture is also used for the calculation of the whole cell volume or the volume of intracellular organellae. $3,150\times$

FIG. 3. Autoradiogram of a part of the interstitial cell. Developed silver grains have been scattered in the membranes of agranular or granular reticulum, and mitochondria. $64,000\times$

FIG. 4. Autoradiogram of the microsomal fractionation of the X-ray irradiated and gonadotropine treated rat testis. Developed silver grains are located in the microsomal membranes. $140,000\times$