Simplified Procedure for the Histochemical Demonstration of Dehydrogenase Activity in Rat Ovaries

To the Editors:

Histochemical techniques for the demonstration of enzyme activity will require the precise localization of enzyme without loss of its activity. In general, non-treated fresh frozen sections do not show a fine histological localization of the enzyme, though the activity is kept at a high level. On the other hand, frozen-substituted sections prepared by the method of Chang and Hori show a fine histological localization of enzyme activity in liver but not in ovarian tissues (Chang and Hori, 1961, 1962a and 1962b; Matsuzawa and Hori, 1963; Matsuzawa, 1963).

For the preservation of enzyme activity and its locality in sections of ovarian tissue, a simplified procedure has been developed in this laboratory.

The present paper deals with the distribution of enzyme activity in tissue sections of rat ovaries prepared by this simplified procedure.

Fresh frozen sections of ovarian tissue from adult rats of Wistar strain were prepared at 10μ thick with a rotary microtome at -20°C. After being dried in air, fresh frozen sections were immersed in cold absolute acetone for 15–20 hrs., then in cold absolute ether for 2 hrs. and warmed up to -20°C. Ether and acetone were refrigerated with solid carbon dioxide (dry ice) during the treatment. These sections treated with cold acetone and ether were transferred to a solution of 85% acetone (-20°C) and dried quickly at room temperature.

For demonstration of enzyme activity, the following staining methods were employed: the method of Chang and Hori for NAD- and NADP-diaphorase (Chang and Hori, 1962a) and the method of Levy et al., with a certain modification for 3β-hydroxysteroid dehydrogenase (3β-ol-DH) (Levy et al., 1959).

A histochemical incubation medium for 3β-ol-DH contains dehydroepiandrosterone as the substrate and Nitro-BT as an indicator.

It was noted on microscopic observation of these sections that the morphological aspects of localization of enzyme activity was more clear than in those of non-treated fresh frozen sections (Figs. 1, 2 and 3). Moreover, it was often observed that section prepared by this procedure showed higher activity of enzymes. This may be due to the fact that the treatment with cold acetone and ether will remove lipid droplets and is therefore likely to prevent the diffusion of enzymes.

Recently, Goldberg and his co-workers (Goldberg et al., 1964) have reported that frozen sections treated with acetone at -80°C for a few minutes were used for histochemical demonstration of 3β-ol-DH in the ovary. In our experiment, the treatment with cold acetone for a few minutes at -20°C did not remove lipid droplets from sections of ovarian tissues sufficiently. As often observed, lipid droplets absorb the dyes produced by enzyme reactions in histochemical reaction mixtures. This absorption of the dye by lipid droplets causes false localization of enzyme activity in tissue sections.

Activities of NAD- and NADP-diaphorase and 3β-ol-DH were demonstrated in interstitial, granulosa, thecal and luteal cells. Lower NAD-diaphorase activity was found

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Fig. 1. NAD-diaphorase (Nitro-BT method). A section treated with cold acetone and ether. ×40.

Fig. 2. NAD-diaphorase (Nitro-BT method). A section treated with cold acetone and ether. ×100.
Outer layers of granulosa cells show higher activity than inner layers of cells.

Fig. 3. 3β-ol-DH (Nitro-BT method.) A non-treated fresh frozen section. ×400.

Fig. 4. NAD-diaphorase (Nitro-BT method). A section treated with cold acetone and ether. ×400.
The activity is located at the apical part of the cytoplasm (Oviductual cells).

Fig. 5. 3β-ol-DH (Nitro-BT method). A section treated with cold acetone and ether. ×400.
The activity is distributed evenly in the cytoplasm of luteal cells.
in thecal cells and poor activity of NADP-diaphorase was observed in granulosa cells. In a follicle, these enzyme activities were higher in outer layers than in inner ones of cells (Fig. 2). The distribution pattern of NAD- and NADP-diaphorase activity in a follicle was quite similar to that of NAD- and NADP-linked dehydrogenase activities, respectively. The strong activity of these diaphorases and 3β-ol-DH in epithelial cells of oviducts was located at the apical part of the cytoplasm (Fig. 4). In striking contrast, these enzyme activities were distributed evenly in the cytoplasm of granulosa, thecal and luteal cells (Fig. 5).

The activities of other enzymes such as ATPase, non-specific esterase, succinate and glucose-6-phosphate dehydrogenase were also preserved. Further investigation is now in progress.

References