Rat Ovarian 3β-Hydroxysteroid Dehydrogenase: Normal Developmental Appearance in Tissue Culture[1] (34500)

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(Introduced by T. N. Harris)

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Although animal cell cultures rarely perform differentiated functions of the tissue of origin, cultures of adult steroidogenic tissues or organs and of fetal adrenals and testes have been reported to have steroidogenic enzymes, steroidogenesis, and endocrine function (1–3). The presence in tissues of activity of 3β-hydroxysteroid dehydrogenase in conjunction with Δ5-4, 3-ketosteroid isomerase is evidence of steroidogenic capability, since these enzymes are essential for the conversion of Δ5, 3β-hydroxysteroids to the corresponding Δ4, 3-ketones in the early biosynthesis of nearly every biologically active steroid (4).

Our early observation that the rat fetal ovary has no demonstrable histochemical activity of the dehydrogenase (5, 6) has been recently confirmed by Schlegel and coworkers (7). Activity of this enzyme in the rat ovary has been shown to be first demonstrable in the interstitial cells at 9 days of age, and in the theca interna at 10 days of age (7, 8). These observations are consistent with previous studies showing that the rat fetal ovary is not endocrinologically active, does not affect the prenatal growth or differentiation of female reproductive tracts, and is uninfluenced morphologically by fetal decapitation (hypophysectomy), or gonadotropin (9, 10).

Ovaries free of adnexal tissue were obtained for tissue culture by microdissection from 21-day fetuses removed by cesarean section from timed-pregnant (11) Sprague-Dawley females, and from 2-day-old female rats. They were minced and grown on coverslips in McCoy's modified medium supplemented with 15% fetal calf serum, glutamine, and antibiotics.

After varying lengths of time in culture, the cells grown on coverslips were stained overnight for activity of 3β-hydroxysteroid dehydrogenase using dehydroepiandrosterone and DPN as substrates, and tetranitro blue tetrazolium as proton acceptor in steroid-containing and steroid-free medium (12). Two observations support the specificity (13) of this histochemical test. Activity of the dehydrogenase was not demonstrable in any cells stained (1) in steroid-free medium; or (2) in steroid-containing medium to which a relatively specific inhibitor of these enzymes, cyano-ketone (2α-cyano-4,4-17α-trimethylandrost-5-en-17β-ol-3-one) had been added (12). In the presence of the steroidal substrate, dehydroepiandrosterone, activity of the dehydrogenase appears in cells cultured for a period corresponding to 9 days of age or older if the ovaries had remained intact in the rat pups (Fig. 1).

Histochemical studies of intact ovaries in 21-day fetuses and of rat pups of varying ages showed no activity of the dehydrogenase when stained in steroid-free or inhibitor-containing medium. In the presence of steroidal substrate, activity of the dehydrogenase first is demonstrable in the interstitial cells at 9 days of age, and in the theca interna at 10 days of age (Fig. 2). Thus, there is a similar time of appearance of the

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dehydrogenase in ovaries in the intact animal and in tissue culture.

The applicability of the present results to other mammalian tissues or enzymes is supported by Picon's study of the rat fetal testis in organ culture (14). The contribution of the fetal testis to the prenatal growth and differentiation of male reproductive tracts has been well established *in vivo* and in organ culture (9, 15). Activity of the dehydrogenase begins in the Leydig cells of fetal testes when these cells differentiate on Day 15 (16, 4). Picon showed that fetal testes explanted on Day 14½ when activity of the dehydrogenase is not demonstrable, or at 15½ or 16½ days, when activity is demonstrable, all have activity in organ culture at a time corresponding to Day 21 (14). She concluded that fetal testes develop and maintain activity of the dehydrogenase in organ cul-

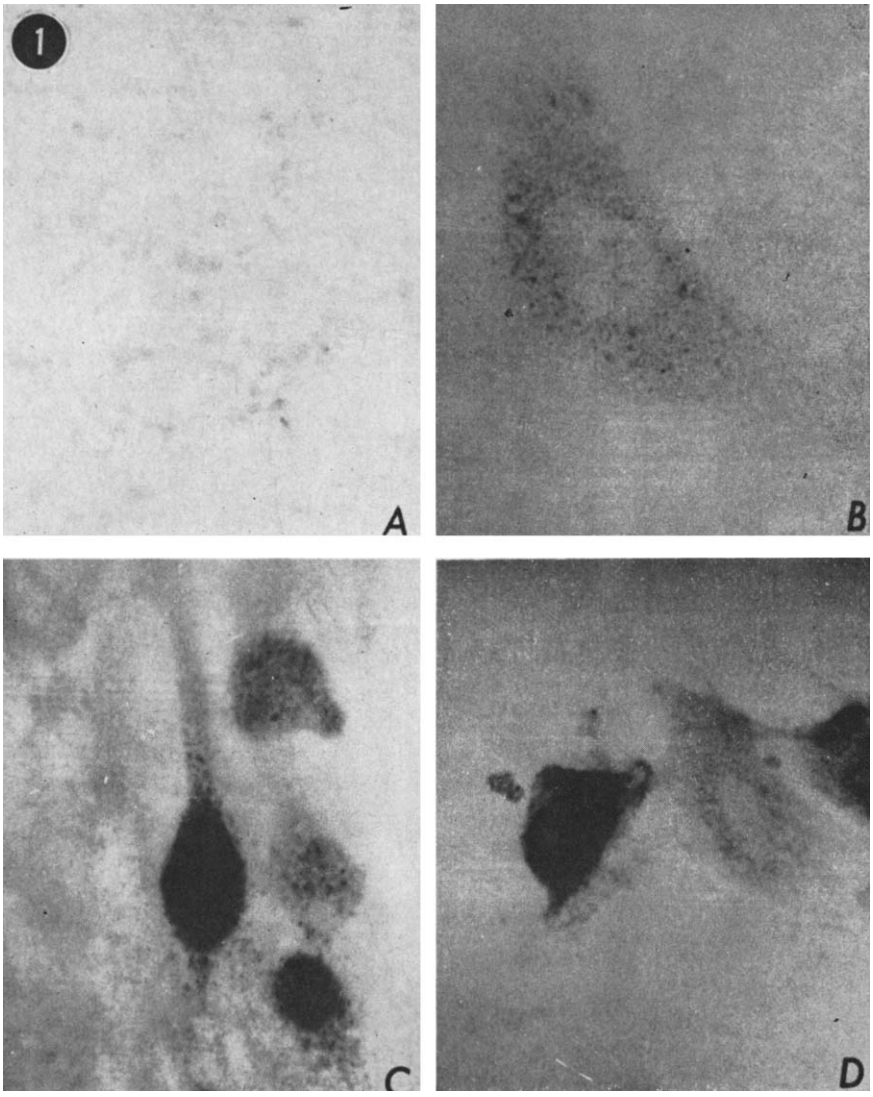

FIG. 1. Histochemical activity of 3β-hydroxysteroid dehydrogenase in cells of fetal ovary grown in culture for a time corresponding to 6 days of age, A (×200); to 10 days of age, B(×900); to 13 days of age, C (×900); and to 16 days of age, D (×900). Note granules representing activity present in B, C, and D, and not in A.

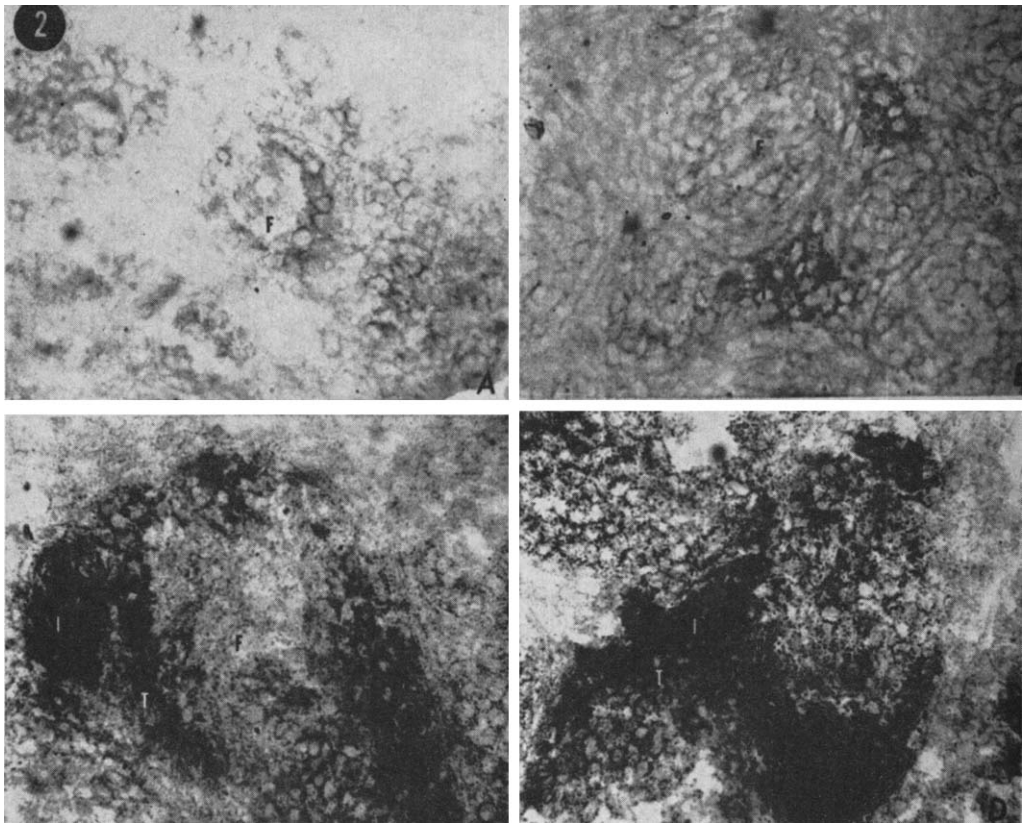

FIG. 2. Activity of 3β-hydroxysteroid dehydrogenase in intact ovaries of pups 6 days old, A; 9 days old, B; 10 days old, C; and 11 days old, D (×900). Note activity beginning in interstitial cells in B, and in theca interna in C. I, interstitial cells; F, follicle; T, theca interna.

ture at a time when they normally do in the fetus.

Summary. Activity of the steroidogenic enzyme, 3β-hydroxysteroid dehydrogenase is demonstrable after 9 days in culture of cells derived from term rat fetal ovaries. Since this is the time at which the dehydrogenase appears in the ovaries of the intact postnatal animal, this enzyme apparently differentiates normally in tissue culture according to a timetable which is inherent in ovarian cells and independent of extraovarian influence. Thus, a model is provided for study of the differentiation of a specific enzyme in mammalian tissue culture, and further suggests that the differentiation of other mammalian enzymes may be sought in tissue culture.

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