demonstrated for leucocytes in lactate, and here exclusively in the carboxyl group[12,13] which corresponds to a fixation of $C^{14}O_2$ via a $C_1 + C_3$ condensation followed by equilibration of the activity of the carboxyl carbons via a symmetrical dicarboxylic acid. Therefore, and in view of the results presented in this communication, the effects of bicarbonate in increasing the activity of the hexose monophosphate pathway would suggest that pyruvate is carboxylated to malate in cooperation with NADPH. Malic dehydrogenase would then substitute NADH for NADPH and the increase of oxygen uptake produced by bicarbonate would be related to reoxidation of NADH through an amytal-sensitive pathway. The relevance of this pathway is demonstrated by the extent of inhibition of the oxygen uptake and of the decarboxylation via the phosphogluconate oxidation pathway found in bicarbonate solution. This amytal-sensitive, i.e. probably flavoprotein, step is not followed by an antimycin- and cyanide-inhibited respiratory chain[2,3].

Recently, WARBURG and his associates claimed that the aerobic glycolysis of leucocytes and other normal tissues should be considered as an artifact due to unsuitable handling, in contrast to the true aerobic glycolysis which would be peculiar to tumor cells[14].

As previously shown in this laboratory[2] and confirmed by others[15], when the procedure followed by WARBURG to avoid damage to the white blood cells is used, the leucocytes obtained are mostly lymphocytes, and aerobic glycolysis cannot be demonstrated with these cells, whilst it is characteristic for polymorphonuclear leucocytes.

With respect to the present experiments, this is a point of considerable interest, since the strong effect of bicarbonate on the aerobic metabolism, as far as the reductive carboxylation of pyruvate is concerned, cannot be produced except in the presence of an active aerobic glycolysis[2].

Riassunto. L'entità del consumo d'ossigeno e della produzione di $C^{14}O_2$ da glucosio-1-C^{14} e glucosio-6-C^{14} e la loro differente sensibilità all'amital nei leucociti polinucleati di essudato incubati in diverse condizioni dimostrano l'esistenza di più sistemi di riossidazione dei coenzimi ridotti.

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The Distribution of some Enzymes Involved in the Steroidogenesis of Hen's Ovary

Evidence for the secretion of sex hormones by the avian ovary has been given by LAYNE et al.[1,2]. Progesterone and oestrogens (oestrone, oestradiol-17β, oestriol) have been identified in the ovarian extracts of the laying hen by chromatographic mobility, UV-spectrophotometry and chemical reactions.

Efforts to identify the cell types in which steroidogenesis takes place have not so far been successful. Only recently Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSDH) has been demonstrated histochemically in the granulosa and the theca interna of the growing oöcytes, atretic follicles and post-ovulation follicles of laying hens (BOTTE[3]). The presence of this enzyme merely indicates the existence of one of the first steps of steroid biosynthesis. In fact, it is demonstrable in all types of steroid-producing cells (adrenal, ovary, testis and placenta) and is involved with Δ^5-3-ketoisomerase in the transformation of pregnenolone to progesterone (see WETTSTEIN[4]). Recently AOSHIMA et al.[5], using a biochemical method, have even demonstrated Δ^5-3β-HSDH in the liver and kidney of the rat.

In this communication, we report the distribution of two enzymes of oestrogen metabolism in the ovary of laying hens (common hen of Aversa): DPN- and TPN-dependent 17β-hydroxysteroid dehydrogenase (17β-HSDH). These enzymes catalyse dehydrogenation of 17β-hydroxysteroids to 17-ketosteroids, and consequently oestradiol-17β to oestrone. They have so far been demonstrated biochemically in the human placenta (LANGER and ENGEL[6], TALALAY et al.[7], HOLLANDER et al.[8], HAGERMAN and VILLEE[9]), and in the liver of amphibia and fishes (BREUER et al.[10]), and histochemically in the human placenta (KELLOG and GLENNER[11], KLEINER et al.[12]) and in the rat liver and intestine (PEARSON and GROSE[13]).

The distribution of both 17β-HSDHs has been compared to that of Δ^5-3β-HSDH on contiguous sections. The histochemical reaction of WATTENBERG[14], modified by LEVY et al.[15], has been used to demonstrate Δ^5-3β-HSDH. DPN- and TPN-dependent 17β-HSDHs have

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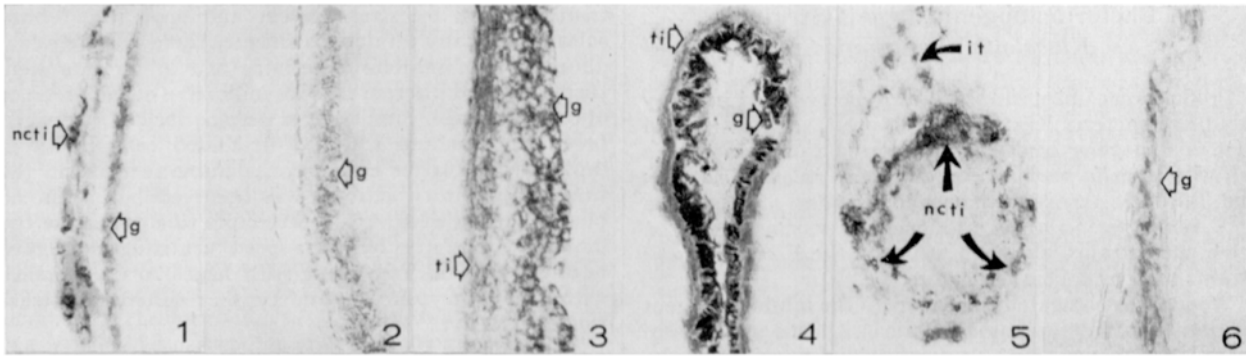

Fig. 1. Cryostat section (15 μ) of the follicle of a growing oöcyte (1 cm in diameter). Reaction for Δ^5-3β-HSDH: the formazan deposit is present in the granulosa cells (g) and the vacuolated cells of the theca interna (ncti). 130 ×.

Fig. 2. Cryostat section of the follicle of a growing oöcyte (1 cm in diameter). Reaction for DPN-dependent 17β-HSDH: the formazan deposit is limited to the granulosa cells (g). 165 ×.

Fig. 3. Cryostat section of the follicle of a fully developed oöcyte. Reaction for Δ^5-3β-HSDH: the activity is consistent both in the granulosa (g) and the theca interna (ti). In the latter the nests of vacuolated cells form a continuous layer. 340 ×.

Fig. 4. Cryostat section of a freshly collapsed follicle. Reaction for Δ^5-3β-HSDH: the activity is much more consistent in the granulosa cells (g) than in the theca interna (ti). 55 ×.

Fig. 5. Cryostat section of an atretic follicle. Reaction for Δ^5-3β-HSDH: the formazan deposit is conspicuous in the vacuolated cells of the theca interna (ncti) and the interstitial cells (it); the granulosa has disappeared. 200 ×.

Fig. 6. Cryostat section of a follicle at the beginning of the atresia. Reaction for DPN-dependent 17β-HSDH: the fine formazan deposit is limited to the granulosa cells. 160 ×.

been characterized at pH 6.9 according to the method of PEARSON and GROSE[13] as adapted by KLEINER et al.[18], using oestradiol-17β as substrate.

The positivity of the above reactions is seen by the precipitation of formazan (reduced tetrazole) granules. Whilst Δ^5-3β-HSDH causes a conspicuous granular precipitation, DPN-dependent 17β-HSDH (TPN-dependent 17β-HSDH is lacking) produces finer deposition of the formazan.

Ovarian follicle. Δ^5-3β-HSDH is detectable in the granulosa and in the nests of vacuolated cells of the theca interna[16] of the growing oöcytes (Figures 1 and 3). DPN-dependent 17β-HSDH is present only in the granulosa cells (Figure 2). TPN-dependent 17β-HSDH is not detectable histochemically in any of the structures of the ovary.

Post-ovulation follicle. Δ^5-β-HSDH activity persists in the granulosa cells and in the nests of vacuolated cells of the theca interna for three to four days after ovulation (Figure 4). Thereafter, activity gradually declines and after seven to eight days all activity disappears. DPN-dependent 17β-HSDH is absent both from the granulosa and the theca interna.

Atretic follicle. Follicular atresia occurs quite frequently in the hen's ovary at any stage of oögenesis and presents varying characteristics. The hypertrophic granulosa cells, which degenerate after the yolk phagocytosis, contain both enzymes, Δ^5-3β-HSDH and DPN-dependent 17β-HSDH (Figure 6).

In the nests of vacuolated cells of the theca interna only Δ^5-3β-HSDH is present. The latter enzyme persists until the more advanced stages of sclerosis of the atretic follicle (Figure 5).

Interstitial tissue. The interstitial tissue is represented by clusters of cells of dubious origin, scattered in the ovarian stroma. They react positively only for Δ^5-3β-HSDH (Figure 5).

In the hen's ovary, then, it is possible to detect Δ^5-3β-HSDH and DPN-dependent 17β-HSDH by histochemical methods. It is interesting to note their different distribution: whilst Δ^5-3β-HSDH is present in the theca interna, interstitial tissue and granulosa either of the pre- and post-ovulation follicle or the atretic follicle, DPN-dependent 17β-HSDH is demonstrable only in the granulosa of the growing ovarian follicle and of the atretic follicle.

These results lead us to suppose that oestrogen metabolism is electively confined to the granulosa cells during follicular growth and during the first stages of follicular atresia. Δ^5-3β-HSDH, detectable in all the steroid-producing tissues, is involved in the secretion of progesterone, which could be further transformed. At this time it is not possible to say, by means of histochemical methods, if further metabolic steps occur.

Our observations, using specific histochemical methods, are an attempt to pinpoint the cell types predominantly responsible for the production of particular hormones in the vertebrate ovary.

Riassunto. È stata studiata con metodi istochimici la distribuzione della Δ^5-3β-idrossisteroide deidrogenasi (Δ^5-3β-HSDH) e delle 17β-idrossisteroide deidrogenasi (17β-HSDH), DPN- e TPN-dipendenti, nell'ovario di gallina ovulante. Mentre la Δ^5-3β-HSDH è presente in tutti i tessuti a secrezione steroide dell'ovario, la 17β-HSDH è localizzata solo nelle cellule della granulosa.

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[16] The theca interna of the growing oöcytes is composed of a continuous layer of connective tissue in which clusters of cholesterol- and fat-laden cells stand out. In the fully developed oöcytes these cells form a continuous layer[17, 19].

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