also studied. In the secondary cultures the greatest H^3-uridine uptake occurred in the first day cells, becoming progressively less pronounced through the second and third days. This event may be associated with gradual nutrient deficiency or overgrowth. Primary cultures showed very little change in uptake from day to day.

Autoradiographic analyses with H^3-pyrimidines revealed that the number of cells containing H^3 grains corresponded to exposure time and was much greater in the secondary cell cultures. Addition of H^3-uridine resulted in silver grains in almost all cells, appearing in 15 min in the nuclei and gradually becoming evident in the perinuclei and the cytoplasmic areas of the cells. This evidence agrees with the general view[8] that the major site of RNA synthesis in the cells is in the nucleus. H^3-thymidine is extensively incorporated into the nuclei.

The elevation of H^3-uridine and H^3-thymidine incorporation into second generation cells is not clearly understood, but it is associated with distinctly different cell sizes between primary and secondary cultures. We have twice successfully transferred aortic intimal cell cultures through the 18th generation. These transferred cells are much larger than first generation cells and somewhat larger than second generation cells (Table). The large cells appearing in second and succeeding generations probably reflect selection or other mechanisms[9] of cellular reaction that are population-dependent and apply to this particular culture environment. How the mechanism is accomplished requires further study.

The data obtained here demonstrate a reproducible method for examining pyrimidine incorporation into chick aortic cells. Analyses of other cellular metabolic activities appear most promising and will be pursued with this type of quantitative technique, using a small amount of cells with labeled precursors[10].

Relation of cell size[a] and culture passage

Generation	Diameter[b] (μ)	Generation	Diameter (μ)
1	10.47 ± 1.10	9	19.21 ± 4.50
2	15.77 ± 1.32	11	20.00 ± 4.83
3	16.37 ± 1.84	13	20.31 ± 4.10
6	16.60 ± 1.10	15	19.22 ± 3.43
7	17.68 ± 2.47	17	22.34 ± 5.42

[a] Measurements were made of the essentially spherical trypsinized cells. [b] Mean and standard deviation of cell diameters of first to third day-old cultures of each generation. More than 600 cells per determination.

Zusammenfassung. Zellen der Kückenaorta zeigten, dass der Einbau von H^3-behandeltem Uridin oder Thymidin in linearem Verhältnis zur Zeitdauer der Stoffeinwirkung stand. Sekundäre, ebenso alle weiteren Zellkulturen ergaben markante Zunahme der linearen Pyrimidinaufnahme, eine Inkorporation, die mit zunehmender Zellgrösse und höherem Stoffwechsel zusammenhängt.

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Loma Linda University School of Medicine, Department of Medicine and Virus Laboratory, Los Angeles (California) and Los Angeles County General Hospital (California USA), June 8, 1965.

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[10] We thank Mrs. T. BRAWNER and Mrs. B. DUKES for technical assistance.

[11] On leave from University of Tokyo School of Medicine, Tokyo (Japan).

Glucose-6-phosphate Dehydrogenase Activity in the Ovaries of *Scomber scomber* L.

The existence of the hexose monophosphate shunt has been reported in many steroid hormone producing tissues of higher vertebrates, including the ovary, adrenal cortex, and testis (FIELD et al.[1]). The glucose-6-phosphate dehydrogenase (G-6-PD) involved in the first step of this alternative pathway is the most important system generating reduced triphosphopyridine nucleotide (TPNH) required for the synthesis of steroid hormones (WHITE et al.[2], HAYNES et al.[3], MCKERNS[4–6], SAVARD et al.[7,8]).

In histochemical studies of human (DEANE et al.[9], KERN-BONTKE[10]) and rat (TUROLLA and MAGRINI[11]) ovaries, it has been reported that the cells considered to be the site of steroid production exhibited a high G-6-PD activity, as well as an activity for Δ^5-3β-hydroxysteroid dehydrogenase. In a previous study on mackerel ovaries (BARA[12]), Δ^5-3β-hydroxysteroid dehydrogenase was localized histochemically and cell types capable of steroid synthesis were identified. It then seemed of interest to examine the mackerel ovaries by histochemical methods used for the demonstration of G-6-PD, for this system is particularly effective in the production of TPNH for steroidogenesis. Techniques for triphosphopyridine nucleotide diaphorase (TPNH-diaphorase) were also applied to the present material.

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The ovaries of sexually mature mackerel were used. Pieces of fresh tissue were promptly frozen, sectioned at 12 μ in a cryostat at – 25°C, and placed on coverslips. Most of the sections were treated with acetone at about – 20°C for 30 min to remove certain lipids. For the demonstration of G-6-PD, the sections were incubated for 15 min at 37°C in a medium (A) designed by COHEN[13], and in a medium (B) prepared according to the method of HESS et al.[14,15]. However, in medium A, 0.1 M phosphate or tris (hydroxymethyl) aminomethane buffer at pH 7.4 was substituted for veronal buffer and, in some instances, tetranitro-BT was substituted for nitro-BT; in medium B, tetranitro-BT or nitro-BT was used, a metal chelate was omitted and the incubation of sections was performed at pH 7.4. For the detection of TPNH-diaphorase, parallel sections were incubated for 15 min in a medium (C) described by COHEN[13], and in a medium (D) prepared according to the method of SCARPELLI et al.[15,16]. The modifications mentioned above for media A and B were also applied to media C and D respectively. Control sections for G-6-PD were incubated in media lacking either substrate or coenzyme, those for TPNH-diaphorase were incubated without substrate. After incubation, the sections were fixed for 10 min in neutral 10% formalin, washed, and mounted in glycerogel.

Although no difference in the distribution of G-6-PD was recorded, the reaction intensity in sections incubated in medium A somewhat exceeded that in sections incubated in medium B.

Ovaries in various stages of the annual sexual cycle were assayed for G-6-PD activity. The details of the stages in the cycle of the ovaries and in the development of the follicles have been described elsewhere (BARA[17]). At the beginning of a new sexual cycle, a distinct G-6-PD activity was found to occur in some of the thecal cells of follicles (Stage 6) in which vitellogenesis had not begun. As follicles at Stages 1–5 presented no enzyme activity, they were not considered here. In ovaries in Stage II, some thecal cells of follicles with yolk vesicles (Stage 7) and of those with lipid droplets (Stage 8) showed a high G-6-PD activity (Figures 1 and 2). It was observed in these ovaries that, beginning from follicles at Stage 6, the number of thecal cells reacting and the intensity of the reaction in these individual cells gradually increased till the follicles reach Late Stage 8. In ovaries in Stage III, a considerable G-6-PD activity was exhibited by certain thecal cells of follicles in which vitellogenesis is well-advanced (Stage 9), while, in ovaries in Stage IV, by those of follicles in late vitellogenesis (Stage 10) (Figure 3) and of follicles at Stage 9. The number of reacting thecal cells, however, was reduced in these follicles. In ovaries in Stages III and IV, while a certain activity was recorded

All Figures show sections of ovaries treated to demonstrate G-6-PD activity. Preincubation in cold acetone. Incubation period 15 min. Nitro-BT. 12 μ. × 240.

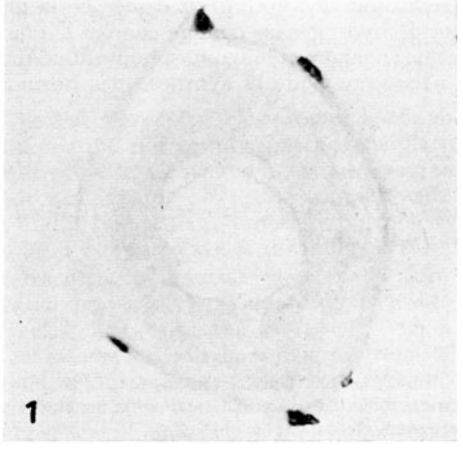

Fig. 1. Section of ovary in Stage II, showing reacting thecal cells (or cell groups) of a follicle at Stage 7.

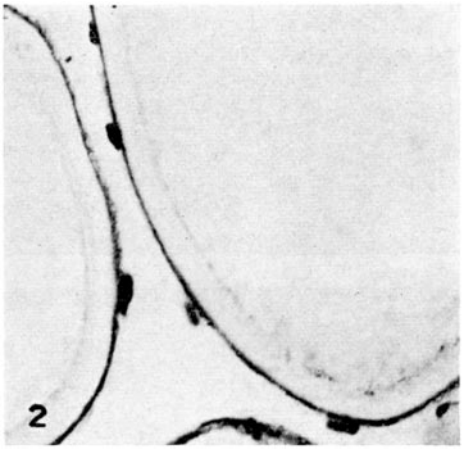

Fig. 2. Section of ovary in Stage II, showing the reaction in thecal and follicular cells of Late Stage 8 follicles. Thecal cells exhibiting a stronger G-6-PD reaction than follicular cells are seen singly or as small groups.

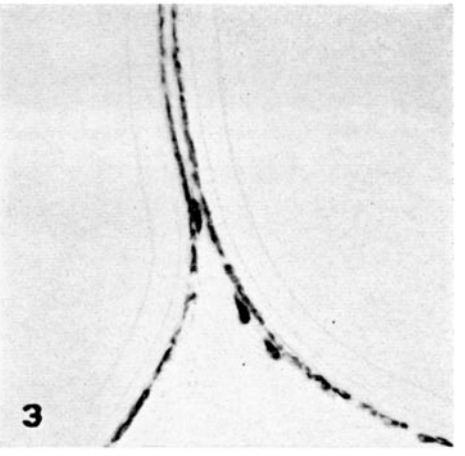

Fig. 3. Section of ovary in Stage IV, showing portions of follicles at Stage 10 with activity in thecal and follicular cells. Note the decrease in the number of reacting thecal cells.

[13] R. B. COHEN, Proc. Soc. exp. Biol. Med. *101*, 405 (1959).
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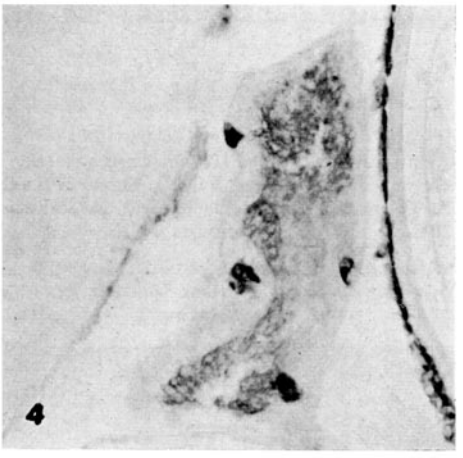
in thecal cells of those at Stage 8, little or none was detected in the thecal cells of the earlier follicles (Stages 7 and 6). In ripe ovaries (Stage V), a few of the thecal cells of mature follicles (Stage 11) as well as of follicles at Stages 9 and 10 reacted positively.

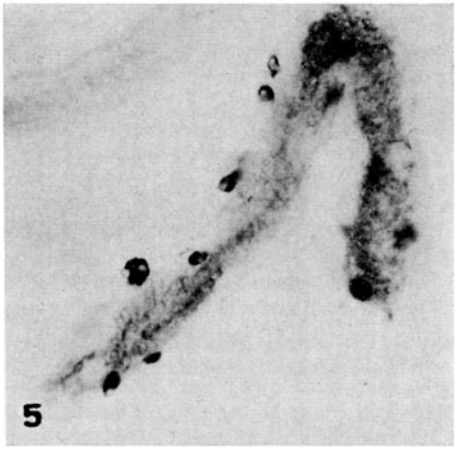
Follicular cells were later than thecal cells in exhibiting a demonstrable G-6-PD activity during the development of the follicles. In follicular cells, a weak G-6-PD activity appeared first in Early Stage 8 follicles found in Stage II ovaries; the activity somewhat increased in follicles at late phases of Stage 8 (Figure 2). In ovaries in subsequent stages, a follicular cell reaction with further increase in intensity was found to occur in follicles at Stage 9, and Stage 10 (Figure 3). A sharp decline in the activity of the follicular cells, however, was observed in mature follicles.

Both in ovaries in a period of preparation for a new shedding after a partial extrusion of eggs (Stage VI), and in ovaries which have just completed final shedding (Stage VII – Early Phase), a high G-6-PD activity was displayed by the follicular cells, and particularly by some thecal cells of spent follicles that were quite new (Figures 4 and 5); whereas the reaction was much reduced in regressed (old) spent follicles. The activity in thecal and follicular cells of the advanced follicles on the onset of degeneration was always less than that of healthy follicles. There was a decline in activity as the degeneration of the follicles proceeded. No G-6-PD activity was detected in structures in late stages of atresia.

In maturing, mature, and spent follicles, it was observed that G-6-PD activity in individual follicular cells was relatively weaker in comparison with the activity in individual thecal cells. In a single follicle, thecal cells reacting positively for G-6-PD occurred as groups of cells or singly. These were larger than unreactive cells in the thecal layer. It has been shown in this study that differences in the G-6-PD activity in thecal and follicular cells occurred in relation to the development of follicles and to the annual ovarian cycle. For ovaries in different stages, it should be noted that the localization of diformazan deposits seen in sections incubated in media used for the demonstration of TPNH-diaphorase was, in general, the same as that observed in sections exposed to media for G-6-PD. The intensity of the staining for the latter enzyme, however, was higher than that for TPNH-diaphorase. In the control sections, no reaction was detected for either enzyme. It was, furthermore, observed that sections incubated in a substrate solution (medium A), in which triphosphopyridine nucleotide had been replaced by diphosphopyridine nucleotide, failed to react.

The present study indicates that G-6-PD revealed a reaction pattern resembling that of Δ^5-3β-hydroxysteroid dehydrogenase (BARA[12]), except that some other structures, including the follicular cells of the maturing follicles found in ovaries in stages before spawning, also reacted. In cells reacting positively to both enzymes, a higher level of activity was observed for G-6-PD after a given incubation period. Although G-6-PD was not restricted to the thecal and follicular cells themselves, the interesting fact is that the G-6-PD activity was high in follicular, and particularly in thecal cells that were previously reported (BARA[12]) to be the only ones to exhibit the activity for Δ^5-3β-hydroxysteroid dehydrogenase which is involved in steroid hormone synthesis. In thecal and follicular cells, the presence of a marked activity for a system that provides TPNH for steroidogenesis supports the role of these cells as the site of steroid hormone synthesis in mackerel ovaries[18,19].





Figs. 4 and 5. Spent follicles in sections of ovaries in Stage VI and in Stage VII-Early Phase respectively. Note that G-6-PD reaction is more intense in thecal cells than in follicular cells and that reacting thecal cells are distributed either singly or in groups in thecal layers of spent follicles. In Figure 4, the reaction in follicular cells of a healthy follicle at Stage 10 (on the right) is also seen.

Résumé. L'activité de la glucose-6-phosphate déshydrogénase a été étudiée histochimiquement dans les ovaires du maquereau. Les cellules thécales et folliculaires du follicule montrent une activité marquée de la glucose-6-phosphate déshydrogénase connue comme le plus important système engendrant la triphosphopyridine-nucléotide réduite, nécessaire pour la synthèse des hormones stéroides.

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