Für genauere quantitative Angaben ist unser Material noch zu klein. Diesbezügliche Untersuchungen sind im Gange.

Diskussion. Entgegen den Angaben von MULNARD[2] haben wir schon während der ersten Segmentationsteilungen in den Blastomeren eine deutlich positive Reaktion auf alkalische Phosphatase gefunden. Eine bilateral symmetrische Verteilung der Reaktionsprodukte in den beiden Blastomeren des 2-Zellstadiums konnten wir weder bei der Reaktion auf DPNH-Diaphorase noch bei derjenigen auf alkalische Phosphatase feststellen. Dies steht im Gegensatz zu den Angaben von DALCQ[3], der eine bilateral symmetrische Verteilung der ATPase in bezug auf die erste Teilungsebene gefunden hat. Ob die polare Verteilung der Reaktionsprodukte in den einzelnen Blastomeren mit den Angaben über die Verteilung mehrerer Dehydrogenasen[4] übereinstimmt, müssen weitere Untersuchungen abklären[6].

Summary. The activity of the DPNH-diaphorase and the alkaline phosphatase were examined in golden-hamster eggs prior to fixation. The reactions for DPNH-diaphorase as well as for alkaline phosphatase were found to be positive already in the undivided egg and in early cleavage stages. The reaction products of both enzyme determinations showed a histophotometrically measurable polar distribution in the cytoplasm, in the one-cell stage as well as in the individual blastomeres of the examined early cleavage stages.

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Anatomisches Institut der Universität Basel (Schweiz), 3. September 1965.

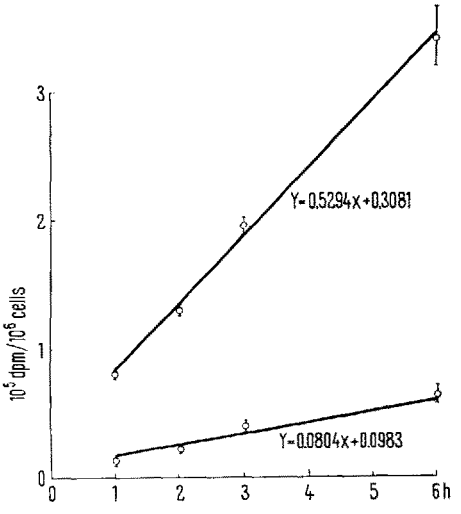
[6] Diese Untersuchungen wurden durch einen Beitrag der Ford Foundation ermöglicht.

Growth of Chick Aortic Endothelial Cells: Incorporation of Tritiated Uridine and Thymidine[1]

Aortic cell culture systems have been utilized to study certain aspects of atherogenesis at the cellular level[2-4]. However, these studies have been principally histochemical. We have attempted to measure cell metabolic rate more quantitatively with radioisotopes added to a small amount of cells in culture. Our experiments have determined cell growth in chick aortic endothelial cell cultures using H^3-uridine and H^3-thymidine. Subcultures of primary lines produced cells characterized by a pronounced acceleration of incorporation of the H^3-pyrimidines and a marked increase in size.

Single intimal cells were obtained by trypsinizing the aortic intimal layers of 3-week-old Rhode Island Red chicks. The cells were resuspended 1:225 (v/v) in Eagle's Minimum Essential Medium (MEM)[5] with 10% fetal calf serum, and planted 5 ml/30 ml tissue culture flask. For secondary and succeeding cell cultures, the cells were transferred by trypsinization after approximately 1 week. Following exposure to 0.2 μc/ml H^3-uridine or H^3-thymidine[6] in MEM with 5% calf serum at 37°C, the sheets of aortic cells were washed 3 times with GKN solution[5] and trypsinized, and cell counts were made. Cells were then dissolved by hyamine to determine the radioactivity by liquid scintillation spectrometer. Autoradiographic studies were also made of the same cell cultures.

The mean H^3-uridine determinations from 6 separate experiments are presented in the Figure. The regression equations[7] of 1-day-old cultures calculated from the experimental values are: primary cultures, $Y = 0.0804x + 0.0983$, and secondary cultures, $Y = 0.5294x + 0.3081$. Highly significant regression lines in both cultures indicate the experiments are reproducible ($P < 0.001$). The calculation also shows there is no deviation from linearity in either regression ($0.2 > P > 0.05$), indicating that the incorporation rate is constant within the 6 h period. The H^3-thymidine uptake rate was essentially the same. The effect of culture age on H^3-uridine incorporation into first and second generation aortic cells, treated identically, was



H^3-uridine incorporation into primary and secondary chick aortic endothelial cells. Points and vertical lines represent means and standard errors.

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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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[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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