and actual functional initiation. It is very interesting to note that the time when the secretory granules appear in the chief cells (the 15th–17th postnatal day period) coincides with the time that lysine, histidine, arginine, tyrosine and isoleucine show an obvious increase in the mucosa (the 15th–17th postnatal day period).

As regards the differentiation period of the chief cells, HELANDER[9] described in his electron microscopic study that the zymogen cells in rat stomach can, with a little doubt, be identified in 20-day-old embryos, by reason of the fact the granules have a larger diameter than those of the mucous neck cells. He also reported that zymogen granules are present in large numbers in the cells at birth, but during the next 10 days they are sparse and that 20 days after birth the zymogen cells have attained a normal adult appearance.

In the author's observations with the light microscope, the rat gastric epithelium consists of columnar cells with long oval nuclei in 20–21-day-old embryos, regardless of the apex or base of the plicae. No cuboidal cells with round nuclei, like the primitive mucous neck cell or primitive chief cell, can be seen. However, sometimes, most of the epithelial cells contain many granules in the apical or supranuclear region of the cells. These granules are secretory granule-like and observable under the light microscope, but the author could not prove that they were zymogen granules.

The figure of the primitive chief cell as seen by the author showed no remarkable change during the differentiation stage, i.e. from the 5th to the 13th postnatal day period. This bears out what HELANDER says, i.e. the zymogen granules in the zymogen cells are few during 10 days after birth, and that peptic activity remains surprisingly constant during the same period.

MABUCHI[4] examined the effect of amino acids on the secretory activity of the chief cells and obtained the result that lysine, histidine, arginine or tyrosine have a promotive effect on the production of secretory granules in the chief cells. The author's findings, i.e. that when these amino acids increase in the gastric mucosa, there is an abundance of secretory granules in the chief cells, may have some connection with MABUCHI's findings. The reason why lysine, histidine, arginine, tyrosine and isoleucine increase from this point onwards has not been ascertained in this study.

Zusammenfassung. Entwicklung und Aminosäuren der Magenschleimhaut an embryonalen und neugeborenen Ratten wurden untersucht und differenziert. In der Magenschleimhaut kann das nicht differenzierte Stadium, das Differenzierungsstadium und das Entwicklungsstadium beobachtet werden. Zahlreiche Aminosäuren nehmen während des Entwicklungsstadiums in der Schleimhaut zu, wohingegen Lysin, Histidin, Arginin, Tyrosin und Isoleucin erst zu einem späteren Zeitpunkt festgestellt werden konnten.

T. NISHIOKA[10]

2nd Department of Anatomy, Wakayama Medical University, 9 Kyuban-cho, Wakayama-shi, Wakayama 640 (Japan), 24 July 1973.

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[10] Acknowledgments. I wish to thank Prof. K. FUJIE for consistent help and excellent suggestions.

PRO EXPERIMENTIS

Demonstration of Synchrony in the Cell Cycle of HeLa Cells by a Flow Microfluorometer

When as asynchronous HeLa cell population is inhibited before the S-phase by a double block of excess thymidine, release of the block would be expected to induce synchronous entry into the S-phase[1,2], This is normally detected by measuring changes in the rates of incorporation of radioactive precursors. However, such methods of monitoring DNA-synthesis are sometimes complicated by pool sizes and require a lot of time. Moreover, the measurement of DNA-synthesis in thymidine-inhibited cells is questionable because it is not quite certain whether the extent of radioactive thymidine incorporation in short pulse periods is a true measure of rates of DNA synthesis[3–5]. These studies were designed to demonstrate the usefulness of a high speed flow microfluorometric method, a technique which is independent of changes in the precursor pool, in synchronization experiments. (See also[6]).

Materials and methods. The investigations were carried out on HeLa S3 suspension cultures during the exponential phase of growth. The cells were grown at 37 °C in 250 ml Sovirell-glass-bottles in 150 ml modified Eagle's minimum essential medium (MEMS, Gibco) supplemented with 2% calf serum (Biocult). A nonheating magnetic stirrer (Bellco) was used to keep the cells in suspension. Cultures were inoculated with 1.7×10^5 cells/ml.

The cells were synchronized by double thymidine treatment, as already described[5,7]. Randomly growing cultures were exposed at 37 °C to 2 m*M* TdR (Serva) for two 12-h periods separated by a 10-h period in normal medium to allow the progression through one S-phase. Thymidine was removed after each 12-h period by centrifugation of the cells at $200 \times g$ for 8 min. The cell pellet was washed with control medium and resuspended in fresh medium. After reverse of the last blockade, 2 ml of cell suspension were pelleted at $200 \times g$ for 5 min and fixed with alcohol-sodium chloride (1:2) with vigorous shaking. The pellet was then washed in 0.1 *M Tris*-HCl buffer (pH 7.5) which was 0.1 *M* in NaCl, and exposed for 1 h to 2% (in the buffer mentioned above) RNase (Serva). After another wash in the same buffer, the cells were resuspended for 30 min in a solution of ethidium bromide (1 mg/100 ml; in *Tris*-HCl, pH 7.5). A 50 µm millipore filter was used to eliminate cell aggregates before measuring fluorescence intensity of DNA-bound dye, using the flow microfluorometer ICP 11 (Phywe AG,

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W. Germany). For control samples of log-phase cells, the procedure was carried out in the same way. With this technique, the measurement of about 20–40,000 cells in one sample is possible within a few minutes. From the DNA-frequency distribution, we obtained the distribution of the cells through the different phases of the cell cycle by accumulation of DNA-frequencies and extrapolation.

Results. Control asynchronous populations in the exponential phase of growth were resuspended in fresh medium (1.4×10^5 cells/ml) and samples were fixed 5 and 11 h later. Figure 1, A–B shows the DNA-distribution patterns with accumulated DNA-frequencies and the calculated percentage of cells in different cell cycle phases in exponential growth cultures. After 5 h the main peak is that of G_1 (40–49%), a second distinct peak is the G_2+M-peak with a 4n DNA-content (30–37%) with the remainder presumably being cells in the S-phase (21–23%). The higher value of G_2+M at 11 h is perhaps

Distribution of cell cycle phases of TdR-blocked HeLa S3 population during a 10-h period of growth in normal medium after release of the block

Time after release of the last TdR-block (h)	G_1 (%)	S (%)	G_2+M (%)
1	85	15[a]	
6	16	84[a]	
7	21	79[a]	
8	12	11	77
9	31	14	55
10	46	13	41

[a] Not calculated for reasons given in the discussion.

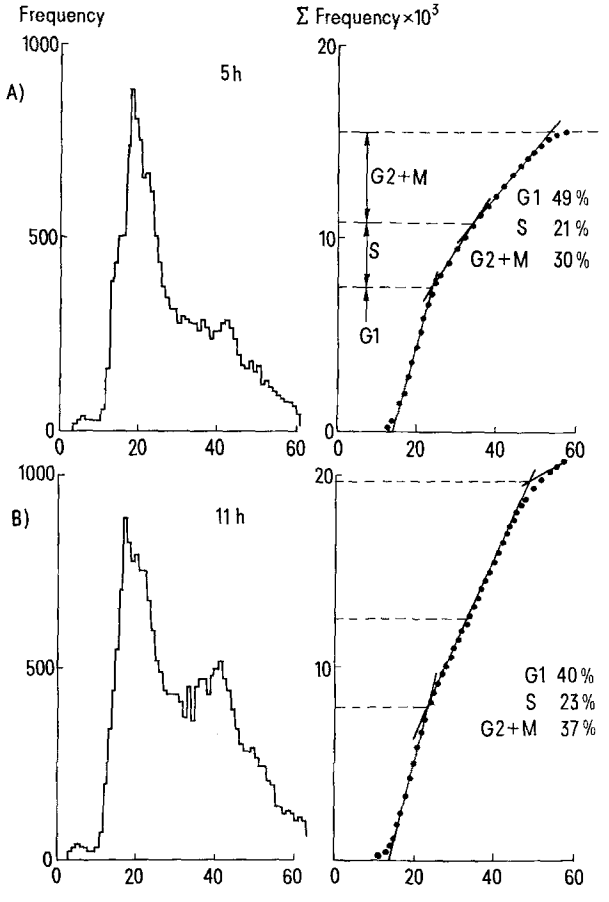

Fig. 1. DNA-distribution pattern and accumulated DNA-frequencies of an exponential-phase HeLA S3 population obtained by measuring the fluorescence intensity using a flow microfluorometer (Phywe ICP 11) of DNA bond dye. A) 5 h after resuspension in normal growth medium. B) 11 h after resuspension in normal growth medium. The calculation of the percentage distribution of the cell cycle phases is based on the graphic approach of the accumulated DNA-frequencies and extrapopulation. Ordinate: Number of cells with equal DNA-content. Abscissa: Relative fluorescence intensity due to DNA-content per cell.

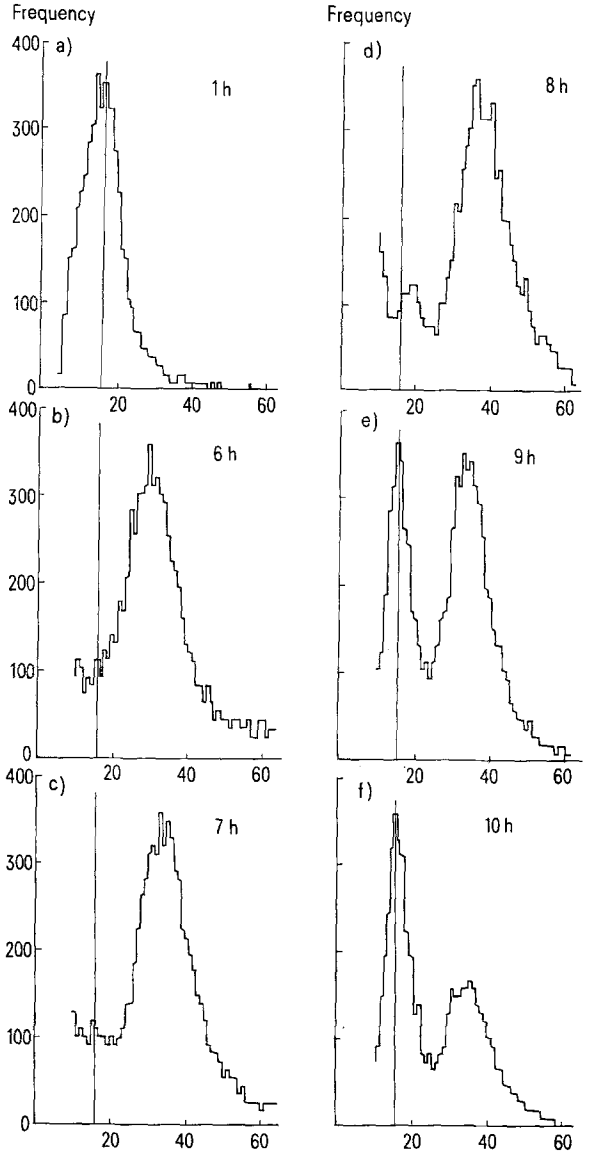

Fig. 2. DNA-histograms of TdR-synchronized HeLa S3 population. Synchronization was carried out with a double block of excess thymidine. Passage through the cell cycle, a-f, was monitored during a 10-h period after release of the block. The vertical lines in all histograms define the position of the G_1-peak in Figure 1. Ordinate: see Figure 1. Abscissa: see Figure 1 and further explanations in the text.

due to a late stimulation effect after the medium change. From cultures synchronized by the double thymidine method, cells were prepared for measuring with the flow microfluorometer at short intervals after release from the last TdR-block. As shown in Figure 2, A–F, cells are in G_1 at 1 h with no detectable G_2+M-phase cells. Over the next few hours it can be seen that whole G_1-cell population synchronously entered the S-phase and subsequently the G_2-phase. The G_1-peak disappeared first but reappeared 8 h after release from the TdR-block, when the cells had gone through mitosis, as was established by counting cell numbers. The Table gives the distribution through the cell cycle phases expressed as percentages.

Discussion. Changes in the DNA-distribution pattern registered by cytophotometric methods were observed in animal cells after treatment with cytostatica[6,8]. Preliminary experiments with HeLa-monolayer cells have indicated that cell synchrony can easily be demonstrated using the methods presented in this report for HeLa-cell suspension.

The synchrony which follows removal of an excess thymidine block is observed as a complete change in the pattern of DNA-distribution within a few hours (Figure 2). Although the cells start from the same point in G_1, a small fraction reaches the next G_1-phase while some cells are still in the first G_2-phase, i.e. there is a gradual decrease in the degree of sychrony, a common observation in synchronized tissue cultures.

No such changes in the pattern of DNA-distribution were observed in asynchronous populations (Figure 1).

The flow of cells through the cell cycle can therefore easily be monitored by changes in ethidium bromide staining of cells. For correct interpretation of these results, one must assume that ethidium bromide binds stoichiometrically to the DNA. The percentage of cells in the S-phase was used to obtain a measure of the degree of synchrony. In earlier studies[9], however, the percentage of S-phase cells in asynchronous populations as monitored by autoradiography was about as high as that obtained from cytophotometric measurements (extrapolation of accumulated DNA-frequencies). These differences can be attributed to the deficiencies in the mathematic method applied. Because of the very small differences in DNA-content of early or late S-phase cells from those in G_1 or G_2+M, difficulties arise in the differentiation of the particular cell cycle phase. No other method (see also[6]) for the processing of results exists at the present time.

Zusammenfassung. Die schnelle fluoreszenzphotometrische Methode wurde zur Bestimmung der Synchronisation des Zellzyklus Thymidin-synchronisierter HeLa-Zellen verwendet. Das Verteilungsmuster der verschiedenen Zellzyklusphasen konnte näherungsweise bestimmt werden.

D. Kübler, M. Stöhr and M. Volm

Deutsches Krebsforschungszentrum, Institut für experimentelle Pathologie, Kirschnerstrasse 6, D-6900 Heidelberg (Federal Republic of Germany),
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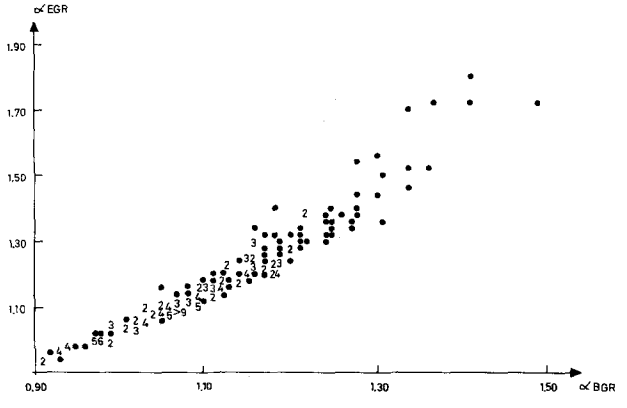
Glutathione Reductase Test with Whole Blood, a Convenient Procedure for the Assessment of the Riboflavin Status in Humans

Erythrocytic $NADPH_2$-dependent glutathione reductase (EGR) has been employed as a parameter for the evaluation of the riboflavin status in humans[1]. For field studies and investigations with children, there is, however, some request to cut down the amount of blood used for the original EGR test, and Thurnham et al.[2] developed a micromethod separating erythrocytes in heparinized capillary tubes.



Stimulation of EGR and BGR for the blood drawn from 203 pregnant women.

Another approach, which has been tried for transketolase by Dreyfus[3], could be the use of whole blood, without separating erythrocytes. The main part of glutathione reductase activity of the blood is in the red cells and, if there is no interference with the assay by substances in the plasma, the procedure seems reasonable.

Recently, the suitability of whole blood for the glutathione reductase test has been shown with rats on a varying riboflavin intake[4]. This communication shows the application of the blood glutathione reductase (BGR) test for humans, and its relation to the EGR test and flavin levels in whole blood.

Materials and methods. Whole blood[5] (stabilized with ACD[6] or heparin) was applied in the BGR test, which differs from the original EGR test[6] mainly in using only 0.05 ml of native or stabilized blood in 9 ml 0.1 *M* phosphate buffer, pH 7.4, enriched with 0.1% EDTA-Na_2; this suspension can be stored frozen for up to several

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