DIURNAL RHYTHM OF MITOTIC ACTIVITY IN LANGHANS' CELLS AND SOME CYTOLOGICAL FEATURES OF THE TROPHOBLAST IN THE HUMAN CHORION

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A cytological study was made of the Langhans' cells in human chorionic villi during the first third of normal pregnancy. Mitotic activity of the Langhans' cells is 2-3 times higher in the evening and at night than in the morning and afternoon. Cytological proof of the cambial role of the cytotrophoblast and of its continuous transformation into the syncytium of the villi was obtained.

Mitotic activity in animals and man has a characteristic diurnal rhythm [1, 2, 5, 13]. However, the provisional organs have not been studied from this standpoint.

The object of the present investigation was to discover whether the Langhans' cells in human chorionic villi have a diurnal rhythm of mitotic activity and to analyze some of the cytological properties of these cells.

EXPERIMENTAL METHOD

A combination of morphological, histochemical, and biometric methods was used to study 130 placentas at successive stages of embryonic development until the end of normal pregnancy, including 98 human chorions at between the 4th and 12th weeks of ontogenesis [4]. These investigations provided morphological and functional criteria for use in the study of chorionic villi at the same level of development. The same principles served as the basis for the present investigation, in which the human trophoblast was investigated cytologically in the first third of normal pregnancy.

Pieces from the chorion were fixed in 10% neutral formalin, in the mixtures of Bouin and Carnoy, and in alcohol-formol. The pieces of tissue were subsequently treated in the usual way and embedded in paraffin wax and celloidin, after which sections were cut to a thickness of 10 μ. The sections were stained with Bömer's, Carazzi's, and Heidenhain's hematoxylins and also with eosin. To analyze mitotic activity [6, 8], the number of mitoses was counted in 6000-10,000 Langhans' cells. The number of dividing cells was expressed as a percentage, and the relative percentages of cells in this group at each phase of division was determined. To discover diurnal rhhthms of mitotic activity in the cytotrophoblast, the results obtained by the study of chorions taken from women undergoing artificial termination of pregnancy in the morning and evening were compared. On the basis of the history, objects identical in all respects were chosen. In addition, nuclei of interphase Langhans' cells were compared karyometrically. The dimensions of the nuclei were studied by means of a drawing apparatus [10]. The numerical results were analyzed by a combination of statistical methods as generally used for cytological investigations [3].

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EXPERIMENTAL RESULTS

The experiments showed that by the end of the first month of intrauterine development the inner layer of the trophoblast in human chorionic villi consists of several layers of large cells containing oval nuclei rich in chromatin. These contain one or two nucleoli with a high RNA content. The entry of Langhans' cells into the syncytium could indicate a cambial role of the cytotrophoblast. The basal part of the Langhan's cells lay on a membrane clearly demarcating the trophoblast from the stroma of the villi. The cytoplasm of the Langhans' cells exhibited oxyphilic properties, unlike the basophilic cytoplasm of the syncytial cover of the villi.

During the 3rd month of pregnancy the nuclei of the cytotrophoblastic cells differed from each other in their distribution of chromatin. The following principal types were distinguished: 1) the chromatin was uniformly distributed throughout the karyoplasm as small granules; these nuclei were strongly chromophilic in appearance; 2) the loose network of chromatin was concentrated by the nucleolus, and the karyoplasm appeared pale and contained a few dark granules; 3) in a smaller number of nuclei the chromatin lay beneath the membrane and the rest of the karyoplasm was almost unstained.

Some of the Langhans' cells were at various stages of mitotic division (Fig. 1). Mitotic activity varied in the different villi, and the coefficient of variation ranged up to 25%. This was evidently because the chorionic villi were at different stages of differentiation. Special comparisons of mitotic activity of the Langhans' cells at different times of day and night showed that in the morning and afternoon the mitotic activity was 1.38% ($m = \pm 0.12$, $\sigma = \pm 0.21$), but in the evening and at night it was significantly higher, viz. 4.32% ($m = \pm 0.54$, $\sigma = \pm 0.92$). A karyometric study of interphase cells of the cytotrophoblast was carried out on the same objects. Variance curves of the mean diameters and volumes of the nuclei were completely identical in type, and as Table 1 shows, the results of statistical analysis indicate a significant difference between the dimensions of chorionic cells obtained in the morning and evening.

The cytological properties of the Langhans' cells described above indicate that these cells penetrate into the outer cover of the villi where they are gradually transformed into a syncytium. This has recently been confirmed electron-microscopically [14, 15]. This pattern of behavior, and the diurnal rhythm of mitotic activity discovered in the Langhans' cells are evidently interconnected and are among the more important properties of the trophoblast. In this connection the views of M. Ya. Subbotin, namely that these results confirm the hypothesis recently put forward by Knorre [9], that the cytotrophoblast is the basal cambium and the trophoblast an extremely specialized epithelium of the cutaneous type, would appear to be correct. The distinctive ratios between the numbers of Langhans' cells in the various phases of mitosis and the absence of karyometric differences among week-old cells probably reflect the existence of a short premitotic phase and a very short phase of mitosis in the trophoblast, together with a prolonged postmitotic period, as other investigations have shown [11, 12].

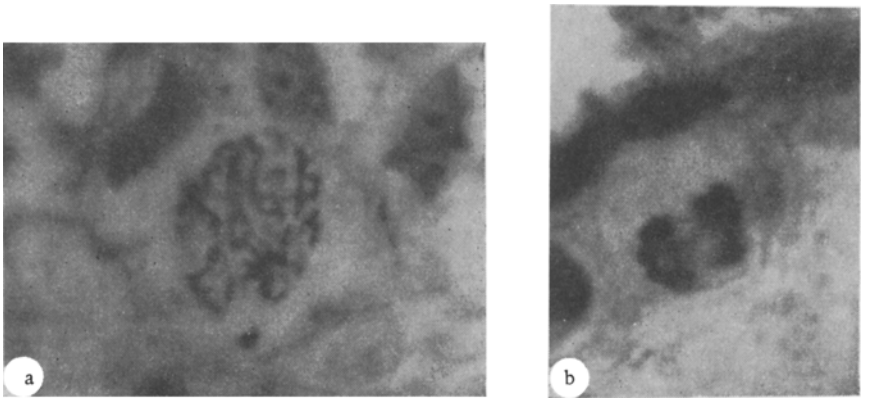

Fig. 1. Langhans' cells in the human chorion. Different phases of mitotic division. Heidenhain's iron-hematoxylin. Photomicrograph: a) prophase, 800× (immersion); b) anaphase, side view. Daughter stars connected by filaments of the spindle, 720× (immersion).

TABLE 1. Dimensions of Interphase Nuclei of Langhans' Cells in Chorionic Villi Obtained in the Morning and Evening

	Statistical index	Morning	Evening
Mean diameter (in conventional units)	M	15,80	15,67
	$\pm m$	0,26	0,14
	$\pm\sigma$	3,60	2,02
Volume of nuclei (in conventional units)	M	2119,0	2080,0
	$\pm m$	49,6	59,1
	$\pm\sigma$	720,0	832,0

Note. One conventional unit in this investigation is equal to 0.416 μ.

The results concerning the diurnal rhythm of mitosis in the human placenta thus emphasize the need for a further study of the periodic activity of the organ linking the mother and fetus, especially with consideration being paid to the varied and complex functions of the placenta. In this way new and valuable facts essential for embryology and the practice of obstetrics could be obtained.

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