CYTOPHOTOMETRIC INVESTIGATION OF THE EFFECT OF ASEPTIC INFLAMMATION ON THE DNA CONTENT IN NUCLEI OF THE RABBIT AMNIOTIC EPITHELIUM

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By cytophotometry at two wavelengths, a marked decrease in the DNA content of the amniotic epithelium of rabbits was found in the early stages of pregnancy. In the late stages of pregnancy this phenomenon is absent. During aseptic inflammation produced by introduction of a silk thread, the number of polyploid nuclei is increased.

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The possibility of multiplication of amniotic epithelial cells in placental mammals is a matter on which different opinions are held. The data so far accumulated are based principally on visual observation of figures of karyokinetic and amitotic division in the amniotic epithelium [2, 4, 7, 10, 11]. There are only a few reports in the literature of studies of the reproductive power of the amniotic epithelium using photometry of nuclei stained by the Feulgen method [5, 6, 12]. The results of a careful photometric investigation of the human amnion at different stages of pregnancy [12] give good grounds for considering that appreciable DNA synthesis takes place in the nuclei in the early stages of pregnancy. Some workers [1, etc.] assert that at the end of pregnancy most nuclei of the amnion are in various stages of pycnosis.

It was therefore interesting to produce aseptic inflammation in the amnion in the later stages of pregnancy and to examine the proliferative capacity of the amnion by the cytophotometric determination of DNA stained by the Feulgen method.

EXPERIMENTAL METHOD

In the late stage of pregnancy laparotomy was performed and a silk thread passed through the amnion under aseptic conditions through the uterine wall. The wall of the uterine cornu was opened 24 h later and a histological and cytophotometric study made of the amnion. Abundant vascularization of the amnion could be seen visually. After removal of the foreign body, a detailed microscopic examination was made of the amniotic epithelium. Changes similar to those described by Dondua and Zavarzin [3] in the amnion of rats with experimental inflammation of the membrane were found in the connective-tissue stroma of the amnion. After removal of the silk thread, the amnion was placed with its epithelial surface externally and impressions of the nuclei were made on a glass slide. Impressions of the nuclei of the amniotic epithelium were made in a similar manner in the late (control) and early stages of pregnancy to discover the dynamics of the DNA content during normal pregnancy. Impressions of the nuclei were fixed in Carnoy's fluid (3 parts absolute alcohol + 1 part glacial acetic acid). These were then hydrolyzed in 1N HCl at 60° for 10 min and stained by Feulgen's method. Cytophotometric investigation was carried out by means of a probe cytospectrophotometer at two wavelengths using Sherudilo's method [9].

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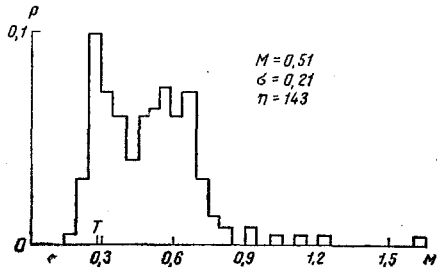

Fig. 1. Histogram of DNA content in nuclei of amniotic epithelium (length of fetus 1.5 cm). Abscissa, DNA content in conventional units (M); ordinate, frequency of finding (P); T represents DNA content in telophase nucleus.

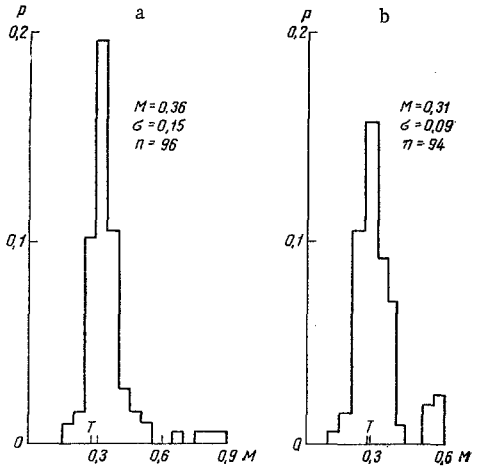

Fig. 2. Histogram of DNA content in nuclei of rabbit amniotic epithelium (length of fetus 7.5 cm). a) Normal; b) 24 h after introduction of silk thread. Legend as in Fig. 1.

EXPERIMENTAL RESULTS

Nuclei, circular in shape and of different sizes, were found in the impressions of nuclei of the rabbit amniotic epithelium at the early stages of development (length of embryo 1.5 cm). Besides interphase nuclei, other nuclei were found in various stages of karyokinetic division. Since DNA synthesis in the nuclei is virtually absent in telophase, the DNA content at this time is constant and corresponds to a diploid set of chromosomes (the number of chromosomes was determined in impressions of nuclei of the amniotic epithelium stained by Feulgen's method). Photometry of the telophase nuclei yielded values for the initial DNA content before the beginning of its synthesis, and this was used when constructing histograms. No mitotic figures were found in impressions of the mature amnion (length of fetus 7.5 cm) in either the control or the experimental series. A noteworthy feature was their smaller size compared with that at earlier stages.

The DNA content per nucleus in the amniotic epithelium of rabbits was increased in the early stages of pregnancy (Fig. 1) by a statistically significant amount ($P = 0.999$ relative to the mature amnion). The criterion λ was used for comparison [8]. The resulting histogram was bimodal because of superposition of two distributions, the first maximum relating to nuclei containing a diploid DNA content, and the second maximum to the tetraploid. The mean DNA content was 0.51 conventional unit, reflecting on the histogram the DNA content in interphase nuclei, while its content in telephase nuclei was 0. 33 conventional unit.

The histograms in Fig. 2 correspond to the normal distribution. A statistically significant increase in the mean DNA content per nucleus (0.36 conventional unit) in histogram a can be attributed to the presence of polyploid nuclei; this can easily be proved by calculating the mean for each case disregarding polyploid nuclei (0.34 conventional unit). The operation led to an increase in the total number of polyploid cells in the epithelium of the mature amnion ($\lambda = 1.63$).

It can thus be concluded from the results of the photometric study of the Feulgen reaction in the amniotic epithelium that appreciable DNA synthesis takes place in nuclei of the rabbit amniotic epithelium in the early stages of development, while in the mature amnion most nuclei of the epithelial cells are diploid; polyploid nuclei are much less frequent, The possibility of their mitotic division is not ruled out, but the reduplication time may be very long and the probability of detection of mitotic figures extremely small.

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