DNA REPLICATION IN AMNIOTIC FLUID CELLS IN CULTURE

Yu. B. Yurov and S. G. Vorsanova

UDC 618.346-008.839.633.2-092.4

KEY WORDS: amniotic fluid cell culture; autoradiography of DNA molecules; DNA replication rate.

Culture of amniotic fluid cells is of great importance for the prenatal diagnosis of chromosomal and biochemical diseases. Methods enabling growth of amniotic fluid cells to be supported highly effectively in culture for a long time (from a few weeks to a few months) have been developed. The morphological features of amniotic fluid cells capable of growth *in vitro* have been characterized in detail. These cells include several types of epithelial-like and fibroblast-like cells [1, 3]. The effectiveness of culture of amniotic fluid cells has been shown to be relatively low, due mainly to the small number of cells capable of growth and colony formation and also to their limited capacity for passage [3]. For example, only in exceptional cases can amniotic fluid fibroblasts in culture withstand several passages [4-6], whereas embryonic and postnatal fibroblasts can pass through about 60 passages on average [7]. Investigations into the cytophysiology of amniotic fluid cells thus indicate that they differ in many properties from other types of cells in culture. The main distinguishing property of amniotic fluid cells is their low ability to grow under conditions of culture *in vitro*. The possible nature of this property of amniotic cells still remains unexplained.

A study of the course of DNA synthesis in cells of higher organisms has shown that the fall in the rate of cell division in culture may be connected with a decrease in DNA replication rate or with an increase in size of the elementary replication units (replicons) [2]. These observations suggested a link between the reduced capacity for growth of amniotic fluid cells cultured *in vitro* and changes in the parameters of DNA replication.

In the investigation described below the method of autoradiography of RNA molecules was used to study the replication rate and dimensions of the replicons in a number of independently obtained primary cultures and subcultures of human amniotic fluid.

EXPERIMENTAL METHOD

All cell cultures used were obtained in the Laboratory of General Cytogenetics, Institute of Medical Genetics, Academy of Medical Sciences of the USSR. The methods used to obtain and culture amniotic fluid cells were described previously [1]. Primary cultures of epithelial- and fibroblast-like cells were grown in Carrel flasks for 10-12 days. Cell lines of amniotic fibroblasts were obtained from primary cultures by subculture in the 2nd-3rd week after seeding and were used in the experiments at the 4th-6th passages. Strains of fibroblasts obtained from the skin of human embryos at the 16th-17th week of pregnancy at the 5th-10th passages were used as the control.

Incubation of the cells with labeled precursors of DNA synthesis began 24 h after replacement of the culture medium by fresh medium (in the case of primary cultures) or after reseeding (in the case of the subcultures). Highly active ^{3}H-thymidine (47 Ci/mmole, 100 μCi/ml) was added first to the flasks for 30 or 60 min, followed by ^{3}H-thymidine with low activity (5 Ci/mmole, 100 μCi/ml) for 90 or 60 min respectively. The methods used to prepare autoradiographs of DNA molecules and to analyze them were described previously [11]. In each case four independently obtained cultures were studied (except the cells of a fetus with anencephaly, in which case only one culture was studied). Determination of the param-

Laboratory of General Cytogenetics, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 9, pp. 349-352, September, 1981. Original article submitted October 22, 1980.

 0007-4888/81/9209-1260$07.50

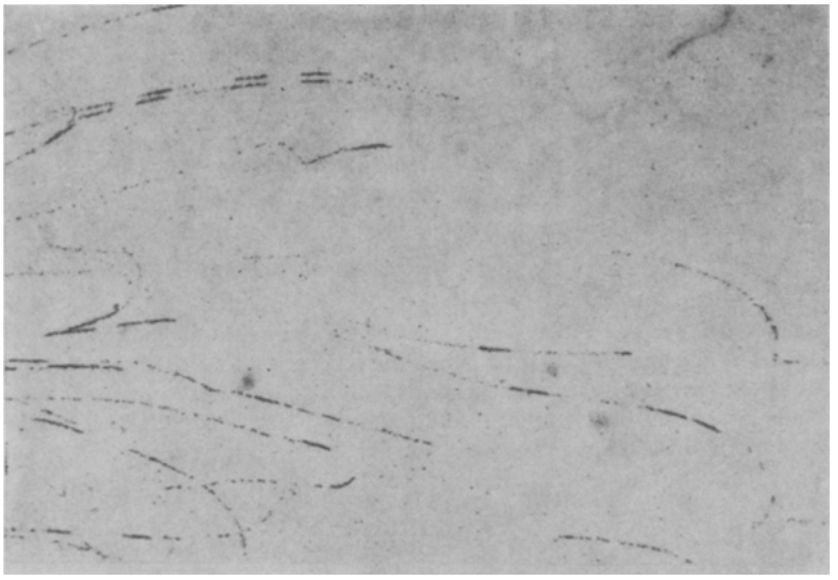

Fig. 1. Autoradiographs of DNA molecules from primary human amniotic fluid cells.

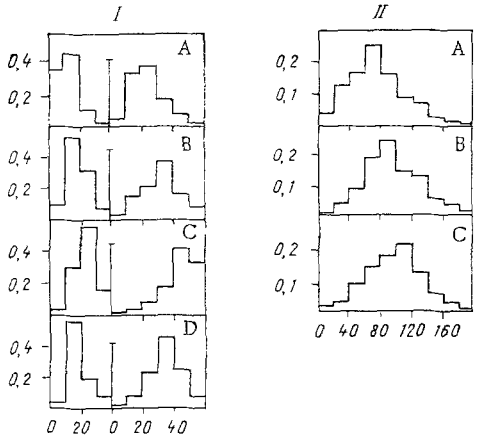

Fig. 2

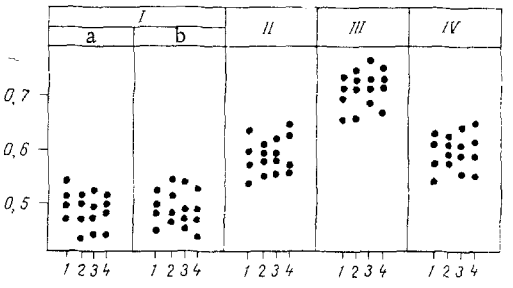

Fig. 3

Fig. 2. Frequency distributions of dimensions of labeled regions of DNA molecules in replication units. I) Dimensions of regions of DNA molecules, labeled with highly active ^{3}H-thymidine for 30 min (left) or 60 min (right) in primary epithelial-like cells (A), primary fibroblasts (B), and subcultured fibroblasts (C and D); II) dimensions of replication units in epithelial-like (A) and fibroblast-like (B) amniotic fluid cells and subcultured fibroblasts from fetal skin (C). Abscissa, length (in μ); ordinate, relative frequency.

Fig. 3. Mean DNA replication rates in amniotic fluid cells and human fetal skin cells. I) Primary cultures: a) epithelial-like, b) fibroblast-like amniotic fluid cells; subcultures: II) amniotic fluid fibroblasts, IV) human fetal skin cells; III) primary cultures of amniotic fluid cells of fetus with anencephaly.

eters of DNA replication was carried out on the basis of 200 measurements for each cell culture. For each type of cells in culture (primary epithelial- and fibroblast-like cells, subcultured amniotic fluid fibroblasts and fetal skin cells) 800 measurements were thus made altogether on the four cultures.

EXPERIMENTAL RESULTS

Examples of autoradiographs of ^{3}H-thymidine-labeled DNA molecules of amniotic fluid cells are illustrated in Fig. 1. The DNA replication rate was determined from the size of

the regions of the DNA molecules intensively labeled by highly active ^{3}H-thymidine for a period of 30 to 60 min (Figs. 2 and 3). A significant decrease in the rate of movement of the DNA replication forks (mean values 0.47 ± 0.02 and 0.46 ± 0.02 μ/min respectively) compared with that in the subcultured fibroblasts from amniotic fluid or human embryonic skin (0.57 ± 0.03 and 0.58 ± 0.04 μ/min respectively) was found. A significant increase in the replication rate on average to 0.71 ± 0.05 μ/min ($P < 0.001$) was found in fibroblast cultures obtained from amniotic fluid cells of the fetus with anencaphaly. The size of the replication units, so far as could be judged from the experimental scheme used, did not differ and varied in all cells studied from 10 to 200 μ, with mean values of the order of 80-100 μ (Fig. 2).

Molecular-cytogenetic analysis of DNA replication patterns in different types of human cells shows that the basic characteristics of their replication units are similar. For example, in differentiated human cells (fibroblasts, heart, brain and liver cells, lymphocytes) and in cells from subjects with various hereditary defects (trisomy for chromosome 21, xeroderma pigmentosa, Fanconi's anemia, cells with the karyotype 45, X; 47, XXX; 49, XXXXV; 49, XXXXX) the velocity of the replication forks and the dimensions of the replicons were virtually indistinguishable [5, 6, 9, 10]. In some hereditary disturbances, however, the normal course of DNA replication may evidently be disturbed. In cells from patients with Blum's syndrome the replication rate was reduced [5], and in cells with trisomy for chromosome 7 it was increased [10]. The increase in the DNA replication rate in anencephaly found in the present investigation is the third case described in which hereditary defects of cells are reflected in the rate of DNA replication. However, in this investigation cells of only one type were tested, and for that reason these data must be regarded as preliminary and requiring confirmation by several independently obtained cell cultures in anencephaly.

There have been isolated reports in the literature on particular features of cells from anencephalic fetuses in culture. A group of workers in Edinburgh found that these cells adhere very rapidly to the substrate, and after a very short adaptation stage they begin to multiply; their rate of multiplication is higher than that of normal human diploid cells [4]. We also noted this property for amniotic fluid cells of the fetus with anencephaly. It can also be postulated that the DNA replication rate in these cells is increased. Consequently, this change may lead to a more rapid rate of cell multiplication in anencephaly *in vitro* compared with the rate of multiplication of normal cells.

The main aim of this investigation was to determine the DNA replication rate and dimensions of the replicons and also to look for a connection between these parameters and the reduced ability to amniotic fluid cells to grow in culture *in vitro*. The results show that the rate of movement of replication forks in primary cultures of amniotic fluid cells is slower than in subcultured cells. These data show that the cytophysiological differences between cells may have an effect on the DNA replication process. It can be tentatively suggested that the low rate of DNA replication in cells of primary (prolonged) cultures is due to the fact that mainly "old" cells, which have passed through many generations in the tissues of the fetus, find their way into the amniotic fluid and, consequently, into culture. Such cells are present in amniotic fluid in the advanced steady-state phase. This hypothesis is supported by the fact that the ability of amniotic fluid cells to multiply for a long time *in vitro*, i.e., to be subcultured, is reduced, in the same way as that of old postnatal cells which have passed through many generations in culture. It should be pointed out that, as experiments with radiography of DNA molecules have shown, the replication rate in "aging" human fibroblasts in late subcultures is reduced by comparison with that in early passages [8]. Our preliminary data show that the rate of movement of the DNA replication forks decreases progressively with an increase in the duration of the stationary phase in human diploid cells aging in the stationary phase. It can be concluded from these observations that DNA replication in aging human cells may take place at a reduced rate.

Sometimes amniotic fluid cells preserve their ability to undergo prolonged culture and may pass through as many as 25 passages. Our observations show that of about 150 independently obtained samples of amniotic fluid cells in culture, only one line capable of prolonged culture could be obtained, and this is being kept in the cell bank of the Institute of Medical Genetics, Academy of Medical Sciences of the USSR. More often, primary cultures can be taken through 3-5 passages, after which they degenerate. These cell lines were evidently obtained from relatively young fetal cells, accidentally shed together with old cells. The basic characteristics of the replication process (velocity of the replication

forks and dimensions of replication units) in these cells capable of successful passage were the same as those in embryonic and postnatal human fibroblasts.

LITERATURE CITED

1. S. G. Vorsanova, Byull. Éksp. Biol. Med., No. 6, 625 (1979).
2. H. J. Edenberg and J. A. Huberman, Annu. Rev. Genet., 9, 45 (1975).
3. A. E. H. Emery, Antenatal Diagnosis of Genetic Disease, Edinburgh (1973).
4. C. M. Gosden, P. Eason, A. Fotheringham, et al., in: Proceedings of the 3rd European Conference on Prenatal Diagnosis of Genetic Disorders, Munich (1978), p. 100.
5. R. Hand and J. German, Proc. Natl. Acad. Sci. USA, 72, 758 (1975).
6. R. Hand and J. German, Hum. Genet., 38, 297 (1978).
7. L. Hayflick, Exp. Cell Res., 37, 614 (1965).
8. T. D. Petes, R. A. Farber, G. M. Tarrant, et al., Nature, 251, 434 (1974).
9. Yu. B. Yurov, Cell Different., 6, 95 (1977).
10. Yu. B. Yurov, Hum. Genet., 43, 47 (1978).
11. Yu. B. Yurov and N. A. Liapunova, Chromosoma, 60, 263 (1977).

EFFECT OF HEAT STRESS ON MORPHOGENETIC POTENTIAL OF NEPHRON EPITHELIUM

O. Z. Mkrtchan

UDC 612.465.014.2-06:612.591

KEY WORDS: kidney; tissue culture; heat stress.

The study of the effect of heat stress on the morphogenetic potential of the nephron epithelium is important for predicting the effects of exposure of the organism and its systems to extremal temperatures. Information in the literature on the effect of heat stress on the excretory system deals with structural and functional shifts [2, 4, 6, 9, 10].

The object of this investigation was to study the action of heat trauma on proliferation, growth, and differentiation of the epithelium of the rat nephron in culture *in vivo*.

EXPERIMENTAL METHOD

Heat stress was produced by keeping animals in a ventilated hot chamber at 45°C for 60 min. The method of organ and tissue culture described by Lazarenko [3] was used. This method gives good results in the study of reactive and plastic properties of the epithelium and tissues of the internal medium and the principles of histogenesis, and for simulating organogenesis and differentiation [3, 11]. This method is being used for the first time to analyze the effect of extremal temperatures on the biological potential of tissues.

Experiments were carried out on 46 male albino rats, in two series: The donors were healthy animals (series I) and animals exposed to heat stress (series II), aged 6 months; the recipients in both series were rats aged 2 months. Under sterile conditions the donors' kidney was removed, decapsulated, and rinsed in physiological saline; the cortex was cut into small pieces measuring 0.67 mm^2, mixed with an equal quantity of neutral celloidin, and implanted subcutaneously into the recipients' anterior abdominal wall. The implants were removed after 1, 3, 6, 8, 10, 15, and 30 days, fixed in Carnoy's fluid, and sections were cut and stained.

Department of Physiology and Anatomy, Tyumen' University. Department of Histology and Embryology, Tyumen' Medical Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR I. V. Toroptsev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 9, pp. 352-355, September, 1981. Original article submitted January 27, 1981.

0007-4888/81/9209-1263$07.50