MECHANISM OF CHANGES IN THE NUCLEOTIDE COMPOSITION OF DNA OF SPERM CELLS PRESERVED IN VITRO

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The nucleotide composition of DNA of viable pig sperm cells remains practically the same as in the spermatozoa of freshly obtained sperm regardless of the time it is kept in vitro. The nucleotide composition of the DNA changes in dying or dead spermatozoa.

Changes in the nucleotide composition of DNA in cells as a result of their aging or treatment with injurious agents have been described [5, 11, 13]. Some workers consider that this is one of the leading factors in the development of the pathological process. However, the methods available for use in isolating DNA from cells for subsequent analysis give an idea of changes in the nucleotide composition of DNA only as an average for the cell, whereas the conclusion regarding these changes usually is related to the whole cell population studied. As a result, despite the undoubted reliability of the results of such investigations, their interpretation in some cases leads to groundless conclusions. These changes in a well-marked form evidently take place only in dying or dead cells.

The object of this investigation was to determine the nucleotide composition of DNA from cells undergoing aging during preservation in vitro. The work was carried out by a method permitting the nucleotide composition of DNA to be determined in cells previously fractionated on the basis of their functional state.

EXPERIMENTAL METHOD

Sperm cells from boars were investigated. The freshly obtained sperm was diluted with 6% glucose solution containing antibiotics and kept at 15°C. Viable (motile) spermatozoa were separated from nonviable (nonmotile, staining with eosin) in sperm samples kept for five days by the filtration method of Parez and Roue [12]. By this method a fraction (filtrate) containing about 90% of viable cells can be obtained from five-day-old sperm containing only about 30% of motile spermatozoa. The cells were defatted and disintegrated and the nucleoprotein isolated by the method of Borenfreund et al. [7], and subsequent deproteinization was carried out by the phenol method [2]. The quality of the isolated DNA preparations was estimated by determining their protein [8] and RNA [6] content and their ultraviolet absorption spectra. The nativeness of the DNA was estimated from the hyperchromic effect [10]. The nucleotide composition of the resulting DNA preparations was determined by descending distributive chromatography on paper [1].

EXPERIMENTAL RESULTS

As Table 1 shows, the ratio between the optical densities at wavelengths of 260 and 280 nm in all DNA preparations was 0.53-0.54, indicating adequate deproteinization of the DNA samples [9]. The protein content in the DNA samples did not exceed 0.6%. The qualitative test for DNA was negative in all the DNA samples. The hyperchromic effect of the DNA of the spermatozoa from freshly obtained sperm was 32.6% and for samples of five-day-old sperm 27.7%, indicating the high degree of polymerization of the DNA in the freshly obtained sperm and some decrease in that degree during preservation of the sperm. The hyper-

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TABLE 1. Comparison of Nucleotide Composition and Some Other Indices of DNA of Spermatozoa from Freshly Obtained Sperm and Filtered and Unfiltered Sperm Kept for Five Days

Index studied	Sperm		
	freshly obtained	kept for five days	
		unfiltered	filtered
E_{280}/E_{260}	0,53	0,54	0,58
Hyperchromic effect	32,6%	27,7%	30,6%
Nitrogenous bases (in moles%):			
adenine	29,99	26,29	29,70
thymine	29,13	27,77	30,40
guanine	19,67	21,37	19,90
cytosine	21,21	24,57	20,42
$\frac{A+G}{T+C}$	0,99±0,03	0,91±0,03 $P<0,05$	0,98±0,02 $P>0,5$
$\frac{A+T}{G+C}$	1,45±0,038	1,18±0,065 $P<0,01$	1,52±0,031 $P>0,5$

chromic effect of DNA of the spermatozoa of the filtered five-day-old sperm occupied an intermediate position, namely 30.6%.

DNA of pig spermatozoa (freshly obtained sperm) is of the well-marked AT-type with a base ratio of 1.45. The ratio between purine and pyrimidine bases obeys Chargaff's rule and was 0.99. During preservation of the sperm this ratio was upset and was reduced to 0.91 for sperm kept for five days. The base ratio was correspondingly reduced to 1.18. The relative content of adenine and thymine in the DNA of the spermatozoa fell during preservation of the sperm whereas the content of guanine and, in particular, of cytosine increased. These changes are evidently not specific. Since the bonds linking the AT pairs are weaker than those linking the GC pairs, it can be supposed that gradual depolymerization of the DNA of the spermatozoa during keeping of the sperm starts somewhat sooner in those parts of the polynucleotide chain which consists predominantly of AT pairs. The results of these experiments agree on the whole with those of other workers [13] who determined the nucleotide composition of DNA of bovine spermatozoa after keeping for 21 days.

However, the determination of the nucleotide composition of DNA of the spermatozoa previously differentiated with respect to their functional state (filtered) led to the conclusion that changes in the nucleotide composition of DNA did not take place in all the cells of the preserved sperm. Changes found in the nucleotide composition of the DNA from the sperm samples kept for five days were due to breakdown of DNA only in the dying or dead cells. It is clear from Table 1 that the nucleotide composition of the DNA from the filtered samples of sperm kept for five days was similar to that of DNA from freshly obtained sperm. The ratio between purine and pyrimidine bases in these DNA samples was 0.98 compared with 0.99 for freshly obtained sperm. The base ratio of the DNA of the filtered samples of sperm kept for five days (1.52) also was very close to the base ratio for DNA of freshly obtained sperm (1.45). Not all the indices of the nucleotide composition of the cell DNA from the filtrate differ significantly from the corresponding indices for DNA from the freshly obtained sperm. However, none of these indices agree exactly. This small disagreement is evidently explained by the fact that the filtration method does not yield a filtrate of sperm after keeping for five days which consists entirely (by 100%) of viable cells. The presence of some degree of nonviable cells contaminating the filtrate causes the indices of the nucleotide composition of the DNA from these sperm samples to deviate slightly from the corresponding indices for freshly obtained sperm.

The nucleotide composition of DNA of viable spermatozoa thus remains unchanged during preservation of the sperm. The observed changes in the nucleotide composition of DNA of the spermatozoa in this period are due to breakdown of DNA in the dying or dead cells. The results of these experiments are in good agreement with earlier observations [3, 4] showing the relative constancy of the DNA content in viable sperm cells preserved in vitro.

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