FURTHER STUDIES ON THE NUCLEAR CHROMATIN OF MORPHOLOGICALLY ABNORMAL BULL SPERMATOZOA

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In ejaculated semen from highly fertile bulls, abnormal head forms constitute 5 to 20% of the total number of sperm heads, while even higher percentages are commonly found in ejaculates from bulls with certain types of reduced fertility. Intranuclear spaces and a granular appearance of the nuclear chromatin were the major abnormalities observed in a high percentage of spermatozoa from two infertile bulls (McCosker, 1969).

The organization of the DNA and nuclear proteins forming the deoxyribonucleoprotein complex (DNP) in the chromatin of deformed sperm heads has received little attention. Previous studies have demonstrated that most morphologically abnormal sperm heads contain the same amounts of DNA as their normally shaped counterparts (Gledhill, 1966a). Defects in the binding of nuclear protein to DNA, which probably occur during the process of chromatin aggregation in spermateliosis, have only recently been incriminated in the formation of certain misshapen sperm heads (Gledhill, Darzynkiewicz & Ringertz, 1971). Their possible rôle in an instance of infertility was noted earlier (Gledhill, Gledhill, Rigler & Ringertz, 1966a).

The present communication reports a study which had as its objective to determine if the DNA of abnormally shaped spermatozoa has a sensitivity to thermal denaturation that differs from that of normally shaped spermatozoa. The method used allows the analysis of molecular arrangements of DNA within intact, definable sperm heads. Such information has physiological importance as it relates to current work on the maturation of nuclear chromatin and could show mechanisms which might be involved in the differentiation of abnormal sperm heads.

Nine sexually mature and normally fertile dairy and beef bulls of varying ages (1½ to 15 years) and breeds were used. Spermatozoa from the testis and from the caput epididymidis were obtained in a manner previously described (Gledhill, 1966b). Spermatozoa were also obtained from the left vas deferens of two of the bulls (courtesy of Dr R. P. Amann, The Pennsylvania State University) at 1 and 3 days after cannulation by a modification of the method of Amann, Hokanson & Almquist, (1963) and Sexton, Amann & Flipse (1971). Ejaculated spermatozoa were collected with the aid of an artificial vagina. The criteria used for differentiating morphologically abnormal from normal

sperm heads were those of Lagerlöf (1934), as adapted for this type of work by Gledhill (1966a).

Thermal denaturation of nuclear DNA *in situ* was measured according to the acridine orange procedure described earlier (Ringertz, Gledhill & Darżynkiewicz, 1970). With this technique, the degree of denaturation is assessed by

TEXT-FIG. 1. Effect of heating on the F_{590}/F_{530} ratio (α) of acridine orange-stained smears of bull spermatozoa obtained from testicle scrapes ($N = 2$ bulls, $n = 20$ cells per experimental variant, i.e. temperature, cell morphology, site of origin), caput epididymidis ($N = 2$, $n = 20$), vas deferens ($N = 2$, $n = 30$) and ejaculates ($N = 3$, $n = 30$). The increase in α reflects the change from a double-stranded to a single-stranded state of the nuclear DNA. Morphologically normal (●) and abnormal (▲) spermatozoa; the S.E.M. is represented by a vertical line.

microfluorimetry of samples previously treated at one of several temperatures between 22° and 100° C. As the incubation temperature increases, the double-stranded DNA is denatured and splits into single-stranded long chain polynucleotides. Since acridine orange binds in a monomer form to double-stranded

DNA giving rise to a green fluorescence, and single-stranded (denatured) DNA binds the dye in an aggregated form giving rise to a red fluorescence, there is a shift towards red in the emission spectrum. By measuring the ratio between the amount of red and green fluorescence ($F_{590}/F_{530} = \alpha$) emitted from individual sperm heads, it is possible to follow changes in the proportion of single- to double-stranded regions induced by heating. A melting-profile of the DNA may be constructed by plotting α against temperature. In the present experiments, an increase in α indicates not only an increased amount of red fluorescence but a reduction in green fluorescence as well since the amount of DNA remains essentially constant.

Following acridine orange staining, the cells were examined in a Zeiss Universal Large Fluorescence Microscope. Excitation light at 365 nm was provided by filtering light from an Osram HBO 100 W/2 mercury lamp through Schott filters BF3, KG1 and BG38. The excitation beam was thereafter directed as reflected light through an F1 reflector. Barrier filters (Schott) for essentially monochromatic light at 530 and 590 nm were interposed between the cells and a RCA-1-P28 photomultiplier. Total fluorescence, registered as scale divisions on a Zeiss Indicator Unit (47-42-05), was corrected for background as described earlier (Gledhill *et al.*, 1966b).

Text-figure 1 shows composite melting-profiles obtained for morphologically normal and abnormal sperm heads. For each site, the similarity of these profiles clearly indicates that there is no difference between normally and abnormally shaped sperm heads in the resistance of their DNA to thermal denaturation. The progressive increase in stability against heat denaturation with advancing maturity reported earlier (Gledhill, 1971) for normally shaped germinal cells again was evident. There was a parallel development of this characteristic in abnormally formed heads.

For samples of both normal and abnormal spermatozoa from the testis and caput epididymidis, the large values for the standard error of the mean at each temperature indicate a greater heterogeneity of response to treatment when compared to spermatozoa from the vas deferens and ejaculate samples. While more information is being gathered on this point (Gledhill & Amann, unpublished data), preliminary data substantiates the premise that molecular alteration of sperm DNP is largely completed in the upper portions of the epididymis.

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