Vitamin A in human semen

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Summary. Vitamin A concentration has been determined in human semen with and without hydrolysis by fluorometry and high performance liquid chromatography (HPLC). The vitamin A content of human spermatozoa is much lower than that reported for rabbit spermatozoa but approximately equal to that of bull spermatozoa.

In the testis there is a specific requirement for vitamin A (retinol) for the maintenance of normal spermatogenesis[2-4]. Vitamin A has been reported to be present in rabbit semen and bull spermatozoa but there have been no attempts to correlate this with functional properties of the spermatozoa[5,6]. Many men with infertility problems have spermatozoa with signs of disturbed integrity of membranes[7,8]. Vitamin A in physiological concentrations is known to stabilize membranes[9]. The present report describes for the first time the identification and quantitation of retinol in the semen of untreated men.

Materials and methods. Semen samples were obtained from men referred to our unit for different andrological disorders and from healthy volunteer medical students. The samples were collected by masturbation after 3–7 days of abstinence. Only specimens assessed as normal in the routine analysis[10] were used. Spermatozoa were obtained by centrifugation of the semen at 1100 × g for 20 min. The seminal plasma was decanted and the pellet washed twice with a buffered salt solution[11]. Vitamin A was analyzed either fluorometrically according to Thompson et al.[12] or by high performance liquid chromatography (HPLC). In the HPLC analysis an internal retinoid standard (RO 12-0586) and ethanol were first added to each sample. The samples were then either hydrolyzed by adding KOH and heating to 80 °C for 15 min or extracted directly with light petroleum without saponification. The extracts were evaporated to dryness, dissolved in methanol, and subjected to HPLC essentially as described by Vahlquist[13]. HPLC quantitation was accomplished by relating the height of the retinol peak to that of the internal standard.

Results. Vitamin A analysis of the saponified seminal plasma by both fluorometry and HPLC yielded similar values. In 6 samples from different men the concentrations ranged from 6 to 20 ng/ml (mean 13 ng/ml). When the samples were extracted without prior saponification the HPLC analysis (retinyl esters not included) showed a mean retinol concentration of only 5 ng/ml, whereas the total vitamin A content was about twice this value. The fluorometric procedure was not sensitive and specific enough to allow a convenient detection of vitamin A in human spermatozoa. On the other hand, analysis by HPLC yielded an obvious peak at the position of all-trans retinol (fig. 1). In a pilot experiment this material was collected from several runs, pooled and assayed fluorometrically. The excitation spectrum (fig. 2) and emission spectrum (maximum 475 nm; not shown) were indicative of retinol.

The amounts of retinol in washed, hydrolyzed spermatozoa from 7 men were 3.8, 6.0, 6.3, 9.4, 15.0, 15.0, and 49 ng/10^8 spermatozoa, respectively. When the spermatozoa were extracted without hydrolysis the retinol values decreased to 5–20% of the values after hydrolysis.

Discussion. Velaquez et al.[6] reported a vitamin A concentration of 3.8 µg/ml in rabbit seminal plasma, which is about 250 times higher than our data for human seminal plasma (13 ng/ml) and 5 times that of normal human blood plasma (appr. 0.7 µg/ml). The vitamin A content of rabbit spermatozoa (2.6 µg/10^8 cells) was also markedly higher than both that of human spermatozoa and of bull spermatozoa (29 ng/10^8 cells)[5,6]. It is not known if the difference between human and bull spermatozoa, on the one hand,

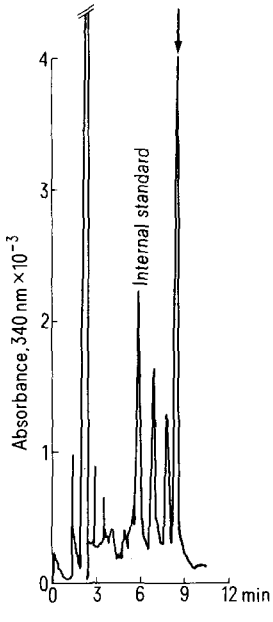

Figure 1. HPLC of a hydrolyzed extract of human spermatozoa. The sample was applied to a Nukleosil 5 µM PEAB-ODS column isocratically eluated with 0.01 M acetate buffer pH 3.6 in acetonitrile (15:85) at a flow rate of 1.2 ml/min. The effluent was monitored at 340 nm with a Waters 440 UV-detector. The arrow indicates the elution position of all-trans retinol.

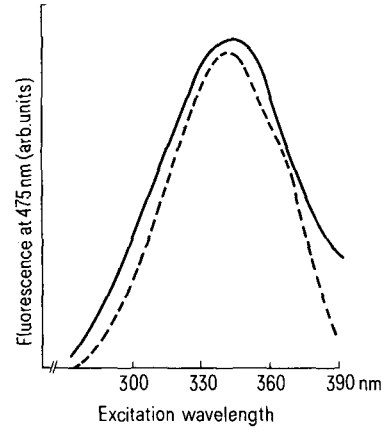

Figure 2. Fluoroscence-excitation spectrum of vitamin A. The sample was dissolved in hexane (500 µl) and assayed in a Turner spectrofluorometer Model 440 with the emission wavelength fixed at 475 nm. ——, Material isolated from HPLC; – – – –, pure retinol (all-trans).

and rabbit spermatozoa, on the other hand, reflects a true species difference or is due to methodological differences. The structural localization of retinol in human spermatozoa was not studied. In rabbit and bull spermatozoa, most of the vitamin A has been postulated to be bound to the acrosome and/or the plasma membrane overlying the acrosome[4,6].

When the hydrolysis of the seminal plasma was omitted the vitamin A concentration was approximately half of that obtained after hydrolysis. When hydrolysis of the spermatozoa was omitted only 5-20% of the retinol was recovered. This may indicate that the vitamin A in the seminal fluid occurs as retinyl esters rather than free retinol and that the vitamin is intimately bound to sperm structures and/or occurs exclusively as a conjugated compound (e.g. retinyl ester).

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Myosin heavy chains in fast skeletal muscle of chick embryo

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Summary. The peptide map obtained by electrophoresis after digestion of purified myosin heavy chains from pectoralis muscle of embryonic chicken with the *Staphylococcus aureus* V8 protease, produces a peptide pattern very similar but not identical to that of adult fast myosin. In fact, some components that are present in a small amount in the map of slow adult myosin are visible in the embryonic pattern.

The data available in the literature about the nature of the myosin(s) in embryonic muscle tissues are still very controversial. In fact, while some authors suggested that in embryonic tissues only 1 type of myosin exists, which closely resembles that of fast skeletal muscle[2-6], it has been demonstrated that developing rat skeletal muscle in embryos or in tissue culture contains a distinct embryonic form of myosin[7-9]. Similar results were obtained in rabbit[10-12] and in chick embryo[13]. In contrast, evidence obtained by means of immunofluorescence studies has indicated on the one hand that early myotubes of the chick can synthesize slow, fast and cardiac myosins[14-17], but, on the other hand, that fetal diaphragm contains only slow-type myosin in addition to fast-type moysin[18]. The discrepancy between these different results may be due to the material examined and the techniques used. In this study, in order to analyze myosin types in immature skeletal muscle more precisely, myosin was purified from the pectoralis muscles of embryonic chickens and the composition of heavy chains was investigated by means of proteolytic mapping of the electrophoretically purified heavy chain portion of the molecule[13].

Materials and methods. The fast twitch pectoralis of 14-day-old embryo Leghorn chicks was studied. For comparative studies myosin was also isolated from the pectoralis, anterior latissimus dorsi (ALD) and the ventricles of adult chicken. Myosin was purified according to the previously reported procedure[19], as specifically detailed by Dalla Libera et al.[20]. Digestion of electrophoretically purified myosin heavy chains was performed according to Rushbrook and Stracher[13], as specifically detailed by Carraro et al.[21], using the V8 protease from *Staphylococcus aureus* as the proteolytic agent (Miles). The following modifications were applied: the thickness of the slab gel was 1 mm; the stacking and the separating gels were 1.6 and 8.5 cm long, respectively. The electrophoretic run was started at 22 mA; 1 h after the tracking dye front had passed in the separating gel, the current was raised to 35 mA. The run was stopped as the tracking dye front reached the bottom of the slab. The amount of protein and protease loaded on each gel is indicated in the legends of the figures. The slabs were stained with Coomassie blue[22]. Densitometric tracings of

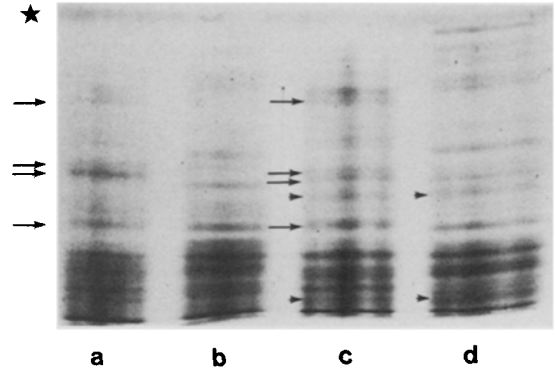

Figure 1. One-dimensional peptide mapping with *Staphylococcus aureus* V8 protease of electrophoretically purified myosin heavy chains from chicken muscles. 20 µg of protein per lane; the *S. aureus* V8 protease/myosin ratio was 1/20. *a* Adult fast pectoralis; *b* adult ventricles; *c* 14-day-old embryonic pectoralis; *d* adult slow ALD. The star locates the *S. aureus* V8 protease band. The bands indicated with arrows are common to embryonic and adult fast maps; the bands indicated by arrowheads are common to embryonic and slow maps. Only the region of the slab under the V8 band is shown.