Polyamines and other accessory sex gland secretions in human seminal plasma 8 years after vasectomy

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Summary. In 56 males, vasectomized 8 years previously, and in 56 age-matched non-vasectomized controls, a number of secretory products of prostatic, seminal vesicular and epididymal/testicular origin were used to monitor post-operative changes in accessory sex gland function.

Significant reductions were observed in seminal plasma volume (3.0 vs 4.9 ml, $P < 0.01$), and the total ejaculate contents of zinc (5.1 vs 9.7 μmol , $P < 0.01$), magnesium (10.6 vs 26.5 μmol , $P < 0.01$), PAP (371 vs 1260 IU, $P < 0.005$) and citric acid (76.7 vs 127.9 μmol , $P < 0.05$), indicating a major impact on secretions of prostatic origin. Unaltered PGE-1 (54.3 vs 53.2 μg , $P > 0.95$) and fructose (3.9 vs 4.5 μmol , $P > 0.1$) indicated no effects on the secretory function of the seminal vesicles.

A marked reduction was demonstrated in the ejaculatory contents of the polyamines, spermidine (366 vs 650 nmol, $P < 0.005$) and spermine (5435 vs 11 804 nmol, $P < 0.05$) but not their acknowledged precursor, putrescine, which is also of prostatic origin.

Keywords: prostate; prostatic secretions; vasectomy; polyamines; man

Introduction

Vasectomy in rats and monkeys causes biochemical changes in the prostate gland (Kinson & Bruce, 1977; Srivastava *et al.*, 1986), an effect which is believed to result from a break in the vascular continuity between the gland and the vas deferens (Pierrepont, 1975). In spite of these published observations, there is a paucity of studies on the effects of vasectomy on accessory sex gland function in the human male. Furthermore, a critical analysis of the world literature revealed that, in the majority of human studies, glandular function was monitored by examining the concentrations of secretory products in the ejaculate (Gregoire & Moran, 1972, 1973; Kothari *et al.*, 1977; Barkan *et al.*, 1978; Nikkanen, 1978; Mendiratta *et al.*, 1980; Naik *et al.*, 1980; Bendvold *et al.*, 1985; Parrish *et al.*, 1987). Seminal plasma is a complex mixture of the products of several glands and it may therefore be more correct to compare glandular function on the basis of their total contributions to the ejaculate. In this way, the confounding effects of alterations in the volumes of secretion produced by the individual glands are avoided. Indeed, vasectomy has been shown to decrease the volume of the ejaculate to an extent which cannot be explained by the absence of the testicular/epididymal contribution (Jouannet & David, 1978; Naik *et al.*, 1980; Nag Das *et al.*, 1984).

The purpose of the present cross-sectional study was to investigate the long-term effects of vasectomy on accessory sex gland function in a carefully controlled study, using the total ejaculate contents of secretory products as indicators of changes in secretory function. The study involved the analysis of many glandular characteristics including putrescine and the polyamines, prostatic products which are also acknowledged to be associated with growth processes in general (Pegg, 1970; Russell, 1970; Chiu & Sung, 1972; Konecki *et al.*, 1975).

Materials and Methods

Study groups. Fifty-six men were investigated at a median of 8 years and 4 months after vasectomy (range 8 years 2 months–9 years 1 month). A control group of equal size was selected with the assistance of the National Population Registry. Every control man was non-vasectomized, married, lived in the same geographic area and was father to at least one child. It was the aim of the study that all control men were born on the same day and year as the corresponding patient. When this condition could not be fulfilled, a person born on a day as close to the patient's birthday as possible was selected. All control men and patients gave their informed consent.

Operation. Vasectomy had been carried out on an out-patient basis under local analgesia with resection of 1–2 cm of the vas deferens via small bilateral scrotal incisions. The resected part of the vas deferens was examined by freeze-microscopy within 30 min after resection and later by light-microscopy of a haematoxylin/eosin stained, formalin-fixed preparation. Azoospermia was confirmed by sperm analysis 2 and 3 months after operation.

Medical history. A careful history of present and previous diseases, medication, and alcohol and tobacco consumption was obtained. Special attention was paid to genito-urinary disorders. The height and weight of each person was measured. Information on sexual activity (frequency of coitus and masturbation) was obtained.

Investigations. A semen sample was provided by each individual at home by masturbation into a condom (PLAN, Danmarks Apotekerforening, Denmark). The condoms contained no chemical addition. The semen samples were all collected after at least 48 h sexual abstinence. Immediately after ejaculation the semen was frozen at -20°C and 3 days later transported frozen to the laboratory where it was transferred to -80°C .

Analysis of semen samples. The samples were thawed. Samples from control men were centrifuged at 1500 g for 10 min at $+4^{\circ}\text{C}$ and the spermatozoa were removed. Seminal plasma volume was measured by weighing on an electronic balance (2 decimal places).

The methods used for the determination of prostatic acid phosphatase (PAP; EC 3.1.3.2), citric acid and fructose have been described previously (Rui *et al.*, 1984), with intra-assay coefficients of variation of 6.3%, 14.3% and 10.8%, respectively. For the determination of zinc and magnesium a Perkin-Elmer model 5000 atomic absorption spectrometer and a microcup flame atomization technique was used. In the assay, 25 μl samples were deproteinized with 10% trichloroacetic acid. Protein was assessed by the method of Lowry *et al.* (1951). Prostaglandin E was determined using a radioimmunoassay kit (Travenol Laboratories Inc., Baxter World Trade Corp., Deerfield, IL, USA: cat. no. CA-501). Testosterone was assessed using a radioimmunoassay procedure described elsewhere (Purvis *et al.*, 1974). Maltase was measured according to Gunaga *et al.* (1967), using 25 μl seminal plasma, a final maltose concentration of 35 mM, and an incubation time of 3 h at 37°C .

Putrescine, spermidine and spermine were determined by a high-pressure liquid-chromatography system (Laboratory Data Control, Riviera Beach, FL, USA) using a procedure essentially like that of Seiler & Knöden (1978). Briefly, 20- μl samples were mixed with 180 μl 1,6-diaminohexane (internal standard; 20 nmol dissolved in water) and deproteinized with 200 μl 0.4 M-perchloric acid on ice for 20 min, followed by centrifugation at 2000 g for 30 min. A sample (25 μl) of the supernatant was diluted with 175 μl water and mixed with 100 mg NaHCO_3 and 0.4 ml dansyl chloride (0.011 M in acetone). The reaction mixture was allowed to stand overnight in the dark at room temperature. Excess dansyl chloride was removed by addition of 0.1 ml L-proline (1.3 M in water) followed by a further 30 min of incubation in the dark. The contents of the tubes then were dried at 65°C under a stream of air, dissolved in 0.1 ml water, and extracted with 0.5 ml toluene. A sample (300 μl) of the toluene phase was evaporated to dryness and dissolved in a final volume of 600 μl methanol. Then 20 μl were applied to the column (250 $\times$ 4.6 mm i.d., Supelcosil LC-18, particle size 5 μm ; Supelco SA, Gland, Switzerland) by an autosampler, and the separation of the dansyl derivatives was obtained using a linear methanol/water gradient changing from 65% to 100% methanol (Ratburn Chemicals Ltd, Walkerburn, UK: h.p.l.c. grade) in 23 min at a flow rate of 1 ml/min. A fluorescence detector with an excitation filter of 340 nm and an emission filter of 460 nm was used, and standards containing 100 pmol spermine, 50 pmol of 1,6-diaminohexane, 10 pmol spermidine, and 10 pmol putrescine per 20- μl injection volume were run after every 7th sample.

Prolactin was measured by a radioimmunoassay using ^{125}I -labelled human prolactin and a second antibody solid-phase technique (Immunobead Reagent, cat. no. 170-5604, Bio-Rad, Richmond, CA, USA). Prolactin values were expressed in international units (50 IU corresponds to 1 mg prolactin, WHO 75/504).

Statistical analysis. Non-parametric methods were chosen due to the abnormal distribution of the data. The Mann-Whitney rank sum test was used for comparison of differences between the two groups.

Results

The median age of men in both groups was 46 years (range 35–70 years). Information on health status revealed no statistically significant difference between the two groups with respect to general health, hospitalization, medication, alcohol and tobacco consumption, height, weight and surface area.

The 2 groups were comparable with respect to sexual activity and duration of abstinence before semen sampling; the median number of ejaculations per month was 8 in both groups (vasectomy group range: 1–30, control group range: 0–60), $P > 0.1$. The mean abstinence period in the vasectomy group was 3.9 days (range 2–8 days) whilst it was 4.7 days (range 2–10 days) in the control group ($P > 0.05$).

Results of the semen analysis are summarized in Table 1. The data are expressed both as concentrations and as total ejaculate contents. In 4 cases (3 controls, 1 vasectomized), the ejaculates were not adequately liquefied after incubation and had to be excluded from the analyses. In other cases, the ejaculate volume was not sufficient to carry out all of the analyses. In these cases the assay of prostatic products was given priority. However, these uneven numbers did not affect the age distribution in the various analyses (Table 2).

Table 1. Accessory sex gland products (concentration and total amount) in seminal plasma from vasectomized men and age-matched control men

Variable	Units	Vasectomized men	Control men	P
Ejaculate vol.	ml	3.0 (0.5–9.6) (55)	4.9 (0.74–16.3) (53)	< 0.01
Zinc	mM	1.76 (0.21–5.75)	2.27 (0.21–5.99)	> 0.05
	μmol	5.10 (1.11–31.85) (41)	9.69 (0.73–38.04) (49)	< 0.01
Magnesium	mM	4.82 (0.29–12.06)	6.17 (1.61–17.7)	< 0.05
	μmol	10.60 (0.71–67.63) (41)	26.46 (3.49–102.69) (49)	< 0.01
PAP	IU/ml	143 (8.7–1277)	298 (35–1653)	< 0.01
	IU	371 (55–7930) (41)	1260 (112–10 496) (51)	< 0.005
Citric acid	mM	26.0 (4–145)	28.8 (4–115)	> 0.5
	μmol	76.7 (5.4–360.8) (42)	127.9 (14–523.9) (52)	< 0.05
Putrescine	μM	71 (0–962)	42.15 (0–1262)	> 0.8
	nmol	202.5 (0–4540) (43)	153.7 (0–4573) (48)	> 0.6
Spermidine	μM	111 (32–1393)	136 (45–570)	> 0.2
	nmol	366 (49.5–8650) (43)	650 (91.8–2806.9) (51)	< 0.005
Spermine	μM	1760 (276–4932)	2566 (244–13 676)	> 0.2
	nmol	5435 (386–36 252) (43)	11 804 (610–69 747) (51)	< 0.05
Fructose	mM	11 (4.4–27.5)	12.2 (1.0–36.6)	> 0.90
	μmol	38.8 (4.8–208.2) (40)	44.7 (3.5–270.8) (51)	> 0.2
PGE-1	μg/ml	25.5 (1.6–110)	18.0 (1.0–84)	> 0.1
	μg	54.3 (1.7–354) (28)	53.2 (3.1–400) (31)	> 0.95
Maltase	units/ml	0.54 (0.10–20.20)	3.48 (0.63–9.03)	< 0.0001
	units	1.42 (0.36–92.92) (26)	11.87 (2.75–40.63) (28)	< 0.0001
Protein	mg/ml	57.0 (36.0–87.4)	55.7 (25.6–91.7)	> 0.5
	mg	282.0 (25.5–835.4) (23)	305.2 (29.6–937.2) (31)	> 0.6
Prolactin	mIU/ml	418 (193–1030)	629 (181–1730)	< 0.05
	mIU	1010 (313–6077) (27)	2128 (489–4500) (29)	< 0.001
Testosterone	ng/ml	0.15 (0.04–1.64)	0.16 (0.04–1.95)	> 0.6
	ng	0.45 (0.11–2.46) (41)	0.69 (0.19–5.85) (51)	< 0.005

Values are median and range with no. of patients in parentheses.

Table 2. Age (median and range) of vasectomized patients and control men subjected to the various analyses of seminal plasma

	Vasectomized men	Control men	<i>P</i>
Zinc and magnesium	46 (35–70)	46 (35–70)	> 0.95
PAP	46 (35–70)	45 (35–70)	> 0.55
Citric acid	46 (35–70)	45.5 (35–70)	> 0.60
Putrescine	46 (35–70)	45 (35–70)	> 0.45
Spermidine and spermine	46 (35–70)	45 (35–70)	> 0.55
Fructose	46.5 (35–70)	45 (35–70)	> 0.45
PGE-1	46 (37–60)	46 (35–60)	> 0.80
Maltase	45 (37–60)	45 (35–60)	> 0.95
Protein	45 (35–70)	45 (35–70)	> 0.60
Prolactin	46 (37–60)	45 (35–60)	> 0.90
Testosterone	46 (35–70)	45 (35–70)	> 0.50

A significant reduction in the volume of seminal plasma was found in vasectomized subjects. When seminal concentrations were compared, a significant reduction in magnesium (4.82 vs 6.17 mM, $P < 0.05$), PAP (143 vs 298 IU/ml, $P < 0.01$), maltase (0.54 vs 3.48 Units/ml, $P < 0.0001$) and prolactin (418 vs 629 mIU/ml, $P < 0.02$) could be detected. When the total ejaculate contents of the secretory products were compared, the majority of secretory products underwent a significant decrease, except for putrescine (202.5 vs 153.7 nmol, $P > 0.6$), fructose (3.88 vs 4.47 μ mol, $P > 0.1$), PGE-1 (54.3 vs 53.2 μ g, $P > 0.95$) and protein (282.0 vs 305.2 mg, $P > 0.6$).

Discussion

The decrease in polyamine content of the ejaculate and the simultaneous reduction in zinc, magnesium, PAP and citrate indicate a major influence of vasectomy on the secretory function of the prostate 8 years after the operation.

The prostate in mammals is the richest source of putrescine, spermidine and spermine (Mann & Lutwak-Mann, 1981). These aliphatic amines are involved in protein synthesis and have many direct actions on DNA- and RNA-polymerases, methylases, hydrolases and actions involving tRNA, rRNA and mRNA in almost all somatic cells (Pegg, 1970; Russell, 1970; Chiu & Sung, 1972; Konecki *et al.*, 1975). It is intriguing that putrescine, a precursor in the biosynthesis of the polyamines, is also not secreted in reduced quantities by the gland after vasectomy. On the other hand, previous studies indicate that, in a variety of physiological situations, the levels of this amine are not often correlated to changes in the other prostatic secretory products (Purvis *et al.*, 1988). For example, frequent ejaculation causes a gradual increase in its secretion, in the presence of decreasing quantities of the other constituents. The paradoxical changes in putrescine may reflect a greater involvement in intracellular biochemical mechanisms other than those involved in secretion, for example, compensatory changes in protein and RNA synthesis.

Zinc, magnesium, PAP, citrate and the polyamines are all acknowledged products of the prostate gland and their seminal plasma concentrations are invariably correlated to each other (Rui *et al.*, 1986). These and other products are secreted by relatively unknown pathways and there is some evidence that they may be produced in different areas of the gland (Hoare *et al.*, 1956; Gyorkey *et al.*, 1967). However, the present study indicates effects of vasectomy on prostate secretory function involving the majority of secretory parameters. This reduces the possibility that the operation has an influence on discrete areas of the gland or on specific biosynthetic pathways. Such generalized effects on glandular function are generally more reminiscent of disturbances in a hormonal influence, for example a reduced effect of testosterone. Indeed, Pierrepoint (1975) postulated that vasectomy can theoretically interfere with the transport of high concentrations of androgens

from the testis to the prostate via the deferential vein. Our own studies and those of others (Adamopoulos *et al.*, 1976; Purvis *et al.*, 1976; Lannon *et al.*, 1980; Ying *et al.*, 1983) suggest that the androgen content of the ejaculate is reduced after vasectomy, which may not just be explained by the absence of epididymal and testicular secretions.

The major reduction in seminal maltase in vasectomized men almost certainly reflects its epididymal origin (Chapdelaine *et al.*, 1978). The same explanation is also relevant for the change in seminal prolactin concentrations, which is primarily derived from the testis/epididymis and also the seminal vesicles (Schoenfeld *et al.*, 1979; Smith & Luqman, 1982; Rui *et al.*, 1985). Both observations confirm previous reports on the effects of vasectomy on maltase and prolactin in the ejaculate (Davis & Lubell, 1975; Chapdelaine *et al.*, 1978; Luqman *et al.*, 1979; Luqman & Smith, 1979; Naik *et al.*, 1980; Smith & Luqman, 1982).

PGE and fructose are primarily secretions of the seminal vesicles and the ampulla of the vas (Schoenfeld *et al.*, 1979; Gerozissis *et al.*, 1982; Sivanesaratnam, 1982; Bendvold *et al.*, 1985). No changes could be detected in their seminal concentrations or their total ejaculate contents after the operation, indicating no major influence of vasectomy on this area of the reproductive tract. Other published reports on these glandular markers tend to be contradictory. Some studies have suggested an influence of the operation on fructose (Naik *et al.*, 1976; Kothari *et al.*, 1977; Nikkanen, 1978), and others no influence on fructose or PGE (Thakur *et al.*, 1975; Srivastava, K. C., *et al.*, 1981; Srivastava, A., *et al.*, 1983; Bendvold *et al.*, 1985). The increase in seminal fructose has been explained by some (Kothari *et al.*, 1977) to reflect a reduced utilization of the sugar in the absence of spermatozoa. In these cases, comparisons were made on the basis of concentrations rather than the total quantity of fructose secreted.

The reduction in seminal plasma volume cannot be explained by the absence of the contribution from the vas deferens, which under normal conditions only accounts for 2–5% of the total ejaculate volume. The specific reduction in the quantities of prostatic materials in the ejaculate implies that the reduction in volume reflects a diminished contribution from this gland. Not only are the specific secretory products of the prostatic epithelium decreased but also the fluid vehicle in which they are secreted.

In conclusion, the results of the present study suggest that vasectomy causes disturbances in prostate secretory function, seen 8 years after the operation. This influence can be best demonstrated when glandular function is assessed using the total ejaculate contents of the secretory products rather than their concentrations. Differences in the expression of the data may explain divergent findings which have previously appeared in the literature. The mechanisms of this influence are at present unknown but may support previous suggestions that there may exist local mechanisms for the transport of high concentrations of androgens from the testis to the prostate (Skinner & Rowson, 1968; Pierrepoint, 1975; Srivastava *et al.*, 1986).

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