

epitestosterone concentrations of 247.4 and 37.1 nmol per 24 h, respectively. As did Oftebro, we concluded that this subject naturally excreted low amounts of epitestosterone. Then we saw the same features in two healthy 15-year-old adolescents with urinary T/E ratios of 7.2 and 6.3.³

The reasons for smaller excretion of epitestosterone in these three subjects are unclear. We first excluded an analytical problem. As required by the Medical Commission of the International Olympic Committee, the T/E ratio is measured by gas-chromatography/mass-spectrometry. The first step of the analysis is hydrolysis of conjugated urinary steroids: testosterone and epitestosterone are excreted as free steroids, glucuronide, and sulphate conjugates.⁵ We used two procedures for hydrolysis: β -glucuronidase from *Escherichia coli* for the glucuronide fraction and methanolysis, which is an efficient way of hydrolysing both steroid glucuronide and sulphate conjugates.⁴ The urinary T/E ratios obtained by the two procedures were similar, which is consistent with the hypothesis that testosterone and epitestosterone present a similar percentage of free, glucuronide, and sulphate fractions.⁵

As mentioned by Kicman and Cowan,⁶ the best explanation of a lower excretion of epitestosterone could be an enzymic deficiency in its biosynthesis. Further studies are required since the metabolism of epitestosterone is poorly documented. It would be necessary to study the frequency of these false-positive doping cases in a larger population and to adopt additional tests for testosterone doping. The ratio of testosterone to luteinising hormone in urine would be a good alternative.⁶

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1. Oftebro H. Evaluating an abnormal urinary steroid profile. *Lancet* 1992; **339**: 941-42.
2. Donike M, Bärwald KR, Klostermann K, Schanzer W, Zimmermann J. The detection of exogenous testosterone. In: Heck H, Hollmann W, Liesen H, eds. *Sport, Leistung und Gesundheit*. Cologne: Deutscher Ärzte Verlag, 1983: 293-300.
3. Raynaud E, Audran M, Pagès JCh, et al. Determination of urinary testosterone and epitestosterone during pubertal development: a cross-sectional study in 141 normal male subjects. *Clin Endocrinol* (in press).
4. Tang WT, Crone DL. A new method of hydrolysing sulfate and glucuronyl conjugates of steroids. *Anal Biochem* 1989; **182**: 289-94.
5. Tajic M, Kovacic E. Free, glucuronic and sulfate testosterone, epitestosterone and androstenedione in the urine. *Naturwiss* 1968; **55**: 394.
6. Kicman AT, Cowan DA. Peptide hormones and sport: misuse and detection. *Br Med Bull* 1992; **48**: 496-517.

Detection of HIV-1 DNA sequences in pre-ejaculatory fluid

SIR,—HIV-1 is generally accepted to be transmissible through sexual intercourse, with seminal fluid as a presumed vector.^{1,2} However, there are no published data on the HIV-1 content of pre-ejaculatory fluid. Since exposure to pre-ejaculatory fluid may occur during sexual intercourse or orogenital contact, this fluid may also be a vector for transmission of HIV-1 infection, if virus is present.

14 HIV-1-seropositive men and 2 seronegative controls provided pre-ejaculatory fluid specimens. HIV-1 DNA was sought by polymerase chain reaction amplification (PCR). Amplified DNA was analysed with a colorimetric, non-radioactive hybridisation assay for the detection of HIV-1 *gag* sequences SK38 and SK39. Assay confirmation was via an oligonucleotide hybridisation assay that used a complementary ³²P-labelled *gag* region probe.

The specimens consisted of 100-500 μ l viscous fluid with white blood cells (WBC) and occasional epithelial cells, but no spermatozoa, although no man was azoospermic. Specimens from 6 of the 14 men (43%) tested repeatedly positive for HIV-1 DNA sequences in pre-ejaculatory fluid. Negative controls tested repeatedly negative. Mean age and serum CD4 counts were not significantly different for men who tested positive or negative for HIV-1 DNA sequences in pre-ejaculatory fluid (*t* test, *p* > 0.05). There was no relation between the rate of HIV-1 detection in men with a history of opportunistic infections (3/4) or symptom-free

CLINICAL DETAILS OF MEN WITHOUT AND WITH HIV-1 IN PRE-EJACULATORY FLUID

	Age	OI/ tumour	Anti- retroviral drugs	CD4 count (/ μ l)	Other known STD
<i>HIV-1 DNA negative</i>					
	47	..	AZT	170	Yes
	27	MAC	AZT	30	Yes
	29	..	..	1210	No
	27	..	AZT	395	No
	29	..	..	530	NA
	34	..	..	740	No
	25	..	..	770	Yes
	33	..	..	650	NA
<i>HIV-1 DNA positive</i>					
	43	..	ddI	61	No
	39	..	AZT	430	Yes
	49	KS	AZT	112	Yes
	29	..	..	1247	No
	36	KS, PCP	AZT, ddI	74	No
	29	..	..	530	No

AZT = zidovudine, ddI = didanosine, KS = Kaposi's sarcoma, PCP = *Pneumocystis carinii* pneumonia, MAC = *Mycobacterium avium-intracellulare*, NA = history of sexually transmitted diseases (STD) not available, OI = opportunistic infection

men (2/10) (*p* = 0.29); nor was there for men on anti-retroviral therapy (4/7) compared with men on no antiretroviral therapy (2/7) (*p* = 0.68, Poisson distribution). 5 men had a history of other documented sexually transmitted diseases (condylomata acuminata 2, gonorrhoea 4, syphilis 3, non-specific urethritis 1). 3 of 6 men without HIV-1 in pre-ejaculatory fluid and 2 of 6 men with HIV-1 had a history of these other sexually transmitted infections (table).

Ejaculated semen is produced by the prostate, seminal vesicles, vasa deferentia, epididymides, and testes, whereas pre-ejaculatory fluid is produced by the urethral (Littré) and bulbourethral (Cowper) glands.³ The role of these glands is unknown, but may be related to prevention of urogenital infection; this is suggested in part by the finding of lymphoid cells in the mucosa of the bulbourethral glands.⁴

Seminal fluid contains measurable amounts of HIV-1 DNA and RNA, thought to be predominantly associated with the WBC fraction as well as cell-free seminal fluid.⁵ The relation of sperm to viral particles remains controversial.⁶ The anatomical source of HIV-infected WBC in seminal fluid is unknown. Our observations point to the presence of WBC in pre-ejaculatory fluid and suggest a potential contribution of this fluid to HIV-1 transmission. Our results indicate that a significant proportion of HIV-1-seropositive men will have detectable levels of HIV-1 DNA sequences in pre-ejaculatory fluid. We were not able to predict reliably who would have HIV-1 in this fluid and until further studies are available, pre-ejaculatory fluid should be considered a potential vector for transmission of HIV-1.

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1. Levy JA. Human immunodeficiency viruses and the pathogenesis of AIDS. *JAMA* 1989; **261**: 2997-3006.
2. Alexander NJ. Sexual transmission of human immunodeficiency virus: virus entry into the male and female genital tract. *Fertil Steril* 1990; **54**: 1-18.
3. Mann T, Lutwak-Mann C. Male reproductive function and semen. Berlin: Springer-Verlag, 1981.
4. Migliari R, Riva A, Lantini MS, Melis M, Usai E. Diffuse lymphoid tissues associated with the human bulbourethral gland. *J Androl* 1992; **13**: 337-41.
5. Anderson DJ, Wolff H, Pudney J, Wenhao Z, Martinez A, Mayer K. Presence of HIV in semen. In: Alexander NJ, Gabelnick HL, Speler JM, eds. *Heterosexual transmission of AIDS*. New York: Wiley-Liss, 1990: 167-80.
6. Marx J. Do sperm spread the AIDS virus? *Science* 1989; **245**: 30.