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## Seminal fluid excretion of cytomegalovirus related to immunosuppression in homosexual men

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### Abstract

Seminal fluid samples from 84 Danish homosexual men were successfully cultured to determine the prevalence of cytomegalovirus excretion. Ten (15%) out of 66 men positive for the antibody were found to be excreting the virus. Although the proportion excreting was inversely related to age ( $p < 0.01$ ), three men aged over 30 and with many years of homosexual experience excreted the virus. In addition, a 50 year old man with Kaposi's sarcoma excreted the virus. A further study of the ratio of T cell helpers to suppressors in the men aged over 30 and a series of age matched non-excreting homosexual control or heterosexual men showed that those excreting cytomegalovirus in their seminal fluid had statistically lower ratios (all  $< 0.77$ ) than the controls ( $p < 0.05$ ).

Excretion of cytomegalovirus may be related to re-emergence of latent infection in immunosuppressed homosexual men.

### Introduction

Homosexual men have a high prevalence of antibodies against cytomegalovirus. In a recent study in San Francisco 94% of 139 homosexual men had antibodies against cytomegalovirus compared with 54% of 70 heterosexual men.<sup>1</sup> Data from Denmark show a similar difference in the prevalence of antibodies against cytomegalovirus between homosexual and heterosexual men.<sup>1a</sup>

Venereal transmission of cytomegalovirus may occur between heterosexuals<sup>2,3</sup> and might explain the high prevalence of antibodies to cytomegalovirus in homosexual men. Cytomegalovirus is excreted in many body fluids, including seminal fluid.<sup>4,5</sup> The frequency of isolation of the virus depends on the age and habits of the men under study. One per cent of men attending infertility clinics were found to be excreting cytomegalovirus in their seminal fluid,<sup>6,7</sup> compared with four out of 64 (6%) of sexually active male university students and men attending venereal disease clinics.<sup>6</sup> Eight attempts to isolate cytomegalovirus from the urine and blood of three of these four men all yielded negative results.<sup>6</sup> Thus seminal fluid may be the only body fluid in which excretion of cytomegalovirus can be shown.

No studies have focused on seminal fluid excretion of cytomegalovirus in homosexual men. Among 190 homosexual men in San Francisco, however, 7% were excreting the virus in their urine compared with none of 101 similarly aged heterosexual men.<sup>1</sup> Studies of cytomegalovirus in seminal fluid have included a few homosexual men—for example, two out of five seminal fluid samples positive for cytomegalovirus in one study were donated by homosexual men, but no information was given about how many homosexual men were studied.<sup>8</sup> In another study of 389 Canadian men nine were known to be homosexual, and three of these were found to be excreting cytomegalovirus in the seminal fluid.<sup>7</sup>

We obtained seminal fluid samples from 101 homosexual Danish men in an effort to establish a more reliable estimate of the prevalence of excretion of cytomegalovirus in this fluid, as it is the most probable source of exposure in homosexual men. In addition, we determined the ratio of T cell helpers to suppressors in selected men to examine the relation between viral excretion and immunosuppression.

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## Subjects and methods

Seminal fluid samples were donated by members of two homosexual communities located in Copenhagen and Aarhus, Denmark. The study had been approved by the local ethics committees in both communities, and all men were volunteers. Within one hour of collection undiluted samples of seminal fluid (0.2 ml) were placed into flasks containing monolayers of human lung fibroblasts with 1-2 ml of medium. After one hour at 37°C to allow for absorption the cells were washed to remove the seminal fluid, which is cytotoxic.<sup>5</sup> Despite this, 17 cultures, including four from men lacking any antibody to cytomegalovirus, did not survive. Flasks were observed over six weeks for the characteristic cytopathic effect of cytomegalovirus, and isolates were confirmed by immune fluorescence methods. Sera from these men were also tested for complement fixing antibody against cytomegalovirus. The methods for culture of cytomegalovirus and antibody testing have been described previously.<sup>8</sup>

For immune studies peripheral blood mononuclear cells were separated and cryopreserved within two to three hours after being collected. Cryopreserved lymphocytes were sent to Bethesda, Maryland, for analysis of T cell subpopulations. Samples were sent in dry ice packing, care being taken to seal the samples away from the vaporising carbon dioxide. The ratios of T cell helpers to suppressors were determined with a fluorescence activated cell sorter according to methods described previously.<sup>9</sup> Monoclonal antibodies used as markers included the 3F10, OKT4, OKT8, and 9.6. These markers were used as indicators of antigens related to HLA, T cell helpers, T cell suppressors, and pan T cell antigens, respectively.

## Results

Out of 84 successful cultures, 18 showed no antibody against cytomegalovirus. As expected, cytomegalovirus was not isolated from these 18 men. Cytomegalovirus was isolated from the seminal fluid of 10 (15%) of the remaining 66 men. Younger men were more likely to be excreting cytomegalovirus than older men ( $p < 0.01$ , Spearman's rank order correlation; table).

The men from Aarhus who were excreting cytomegalovirus in their seminal fluid ranged from 18 to 27 years old and, with one exception, had had relatively little homosexual experience (one, two, two, two, four, and 13 years). By contrast, the men from Copenhagen who were excreting cytomegalovirus were considerably older (26, 32, 36, and 39 years). They reported many years of homosexual activity (seven, 18, 21, and 10, respectively) and many different sexual partners a year (40, 15, 10, and 50 respectively), and had probably sustained the primary infection long ago. Excretion of cytomegalovirus correlated inversely with years of homosexual experience ( $p < 0.07$ , Spearman rank order correlation). Only two men, one in Aarhus and one in Copenhagen, admitted having used nitrite inhalants in the past 12 months.

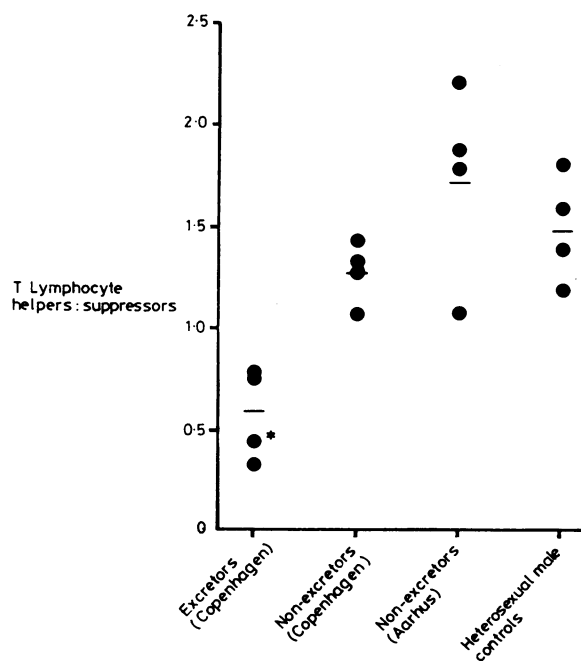
### Excretion of cytomegalovirus in seminal fluid of Danish homosexual men

Age (years)	No of samples obtained	No of cultures that died	No of cultures that survived	No of patients with antibodies to cytomegalovirus	No (%) excreting cytomegalovirus
<i>Copenhagen</i>					
<20	0				
20-29	12	2	10	8	1 (13)
30-39	22	1	21	17	3 (18)
>40	11	1	10	9	
?	3		3	3	
All ages	48	4	44	37	4 (11)
<i>Aarhus</i>					
<20	3		3	3	2 (67)
20-29	30	8	22	15	4 (27)
30-39	12	2	10	7	
>40	4	1	3	3	
?	4	2	2	1	
All ages	53	13	40	29	6 (21)
<i>Total</i>					
<20	3		3	3	2 (67)
20-29	42	10	32	23	5 (22)
30-39	34	3	31	24	3 (13)
>40	15	2	13	12	
?	7	2	5	4	
All ages	101	17	84	66	10 (15)

In the 10 samples positive for cytomegalovirus excretion ranged from one to 50 000 plaque forming units per 0.2 ml aliquot and did not correlate with age. In the three men aged over 30, titres were 1000, one, and 50 plaque forming units per 0.2 ml aliquot.

In addition we cultured a sample of seminal fluid from a 50 year old homosexual with Kaposi's sarcoma and persistent immunosuppression, although he had never been treated with immunosuppressive drugs or radiation. Details of his condition have been reported previously.<sup>10</sup> He was also found to be excreting cytomegalovirus (10 plaque forming units per 0.2 ml aliquot).

To determine whether more experienced homosexual men who were excreting cytomegalovirus in their seminal fluid samples were immunocompromised we evaluated the ratio of T cell helpers to suppressors in the following subjects: the three men aged over 30 who were excreting the virus; the 50 year old patient with Kaposi's sarcoma (also excreting); four homosexual men from the same community and four homosexuals from a different community matched for age who were not excreting the virus; and four heterosexual male controls in the same age range. The figure shows the results. The difference in the ratio between the homosexual men aged over 30 who did and did not excrete the virus was significant ( $p < 0.05$ , Student's *t* test, two-tailed). Although it would have been of additional interest to examine the ratio of T cell helpers to suppressors in the 27 year old man from Aarhus who was excreting cytomegalovirus despite 13 years of homosexual experience, he was not available for sampling.



Ratios of T lymphocyte helpers to suppressors in healthy Danish homosexual men who either were or were not excreting cytomegalovirus in their seminal fluid, and in heterosexual male controls. (Bars indicate mean values.)

\*Patient with Kaposi's sarcoma.

## Discussion

These results indicate that excretion of cytomegalovirus in the seminal fluid of homosexual men is common and related to age. This relation with age has been observed in studies of excretion of cytomegalovirus in the urine of women<sup>11</sup> and men<sup>1</sup> and has been attributed to recent primary infection in those donating samples. Excretion of cytomegalovirus in younger homosexual men, particularly those who have recently initiated a homosexual lifestyle, might be expected if they are examined soon after contracting a primary infection. In contrast to other studies, however, in which all people excreting the virus were aged under 30, we found that three of the four homosexual men from Copenhagen who were excreting the virus were over 30.

All three had had many years of homosexual activity and many different sexual partners a year, and therefore their primary infections had probably occurred many years earlier. Our finding of excretion of the virus in these men might indicate that some homosexual men continue to excrete cytomegalovirus in seminal fluid over many years or, alternatively, that there had been recent reinfection, perhaps with a different strain of cytomegalovirus. More probably, however, latent cytomegalovirus re-emerges in men with immunosuppression.

Recently there have been many reports of homosexual men developing illnesses, including Kaposi's sarcoma, that are related to immune suppression. Clinical and epidemiological aspects of these illnesses were recently summarised.<sup>12</sup> Evidence of abnormal ratios of T cell helpers to suppressors in apparently healthy homosexual men has also been documented,<sup>13</sup> and the low ratios reported are similar to those that we found in the older homosexual men who were excreting cytomegalovirus in their seminal fluid. Excretion of cytomegalovirus in the seminal fluid of these more experienced homosexual men and of a homosexual patient with Kaposi's sarcoma is therefore probably the result of immune dysfunction.

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# Schistosomiasis mekongi diagnosed by rectal biopsy

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## Abstract

*Schistosoma mekongi*, a parasite of the dog which is endemic in small foci along the Mekong river, is rarely seen in Europe. A case of schistosomiasis caused by *S mekongi* was recently seen in France, in a native of Laos who had been living in Thailand. He was admitted for treatment of previous diagnosed tuberculoid leprosy but was found to have hepatosplenomegaly, with a high eosinophil count and high bilirubin concentrations. Serological examination suggested schistosomiasis, but there were no parasites in the urine and the rectal mucosa looked normal on endoscopy. Nevertheless, rectal biopsy showed schistosomal proctitis and many ova of *S mekongi*. Two courses of praziquantel cleared the ova from his rectal mucosa.

Although serological examination using *S mansoni*

antigen suggested schistosomiasis, only biopsy of normal looking rectal mucosa allowed the diagnosis to be established.

## Introduction

*Schistosoma mekongi* is a parasite of the dog. It is endemic in small foci along the Mekong river in Laos and Thailand.<sup>1</sup>

## Case report

A 21 year old man was admitted for the supervision of his tuberculoid leprosy, diagnosed two years before, and treated with sulphones (dapson). He was a native of Vientiane (Laos), but had lived in Thailand for two and a half years before his arrival in France. He complained of right hypochondrial pain and had slight conjunctival jaundice. Clinical examination showed hepatosplenomegaly, the spleen extended beyond the costal rim by 3 cm. Blood examination showed hyper eosinophilia ( $8.96 \times 10^8/l$ ). The total bilirubin concentration was high at  $26 \mu\text{mol/l}$  ( $1.5 \text{ mg}/100 \text{ ml}$ ) with a ratio of total to conjugated bilirubin of 7:1. Examination of the faeces showed some eggs of *Opisthorchis viverrini* and some larvae of *Anguillula*. A serodiagnosis of schistosomiasis was made after an indirect immunofluorescence technique showed a positive response to the antigen *S mansoni* at a titre of 1/40. There were no parasites in the urine. Rectal endoscopy showed no abnormal changes, but histological examination of two rectal biopsy specimens taken from normal looking rectal mucosa showed lesions of schistosomal proctitis and the presence of

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