

- 4 Semprini AE, Persico T, Thiers V, et al. Absence of hepatitis C virus and detection of hepatitis G virus/GB virus C RNA sequences in the semen of infected men. *J Infect Dis* 1998; **177**: 848–54.
- 5 Wejstal R. Sexual transmission of hepatitis C virus. *J Hepatol* 1999; **13**: S92–95.

Laboratoire de Virologie, CHU Necker-Enfants Malades, Paris, France (M Leruez-Ville MD, C Rouzioux PhD, M-L Chaix MD); and **Laboratoire de Biologie de la Reproduction, CHU Cochin-Port Royal, Paris, France** (J-M Kunstmann MD, M de Almeida MD)

Correspondence to: Dr Marianne Leruez-Ville, Laboratoire de Virologie, CHU Necker-Enfants Malades, 149 rue de Sévres, 75015 Paris, France (e-mail: marianne.leruez@nck.ap-hop-paris.fr)

How reliable is a vasectomy? Long-term follow-up of vasectomised men

N Haldar, D Cranston, E Turner, I MacKenzie, J Guillebaud

Around 42 million couples worldwide rely on vasectomy as a method of family planning. It is well recognised that a vasectomy can fail at any stage, and therefore warning couples of risk of failure forms an important part of the consent procedure.

In most cases azoospermia is achieved within 4 months of vasectomy. Early failure is noted when the patient is not clear of sperm at the first semen analyses. Late failure, with the presence of sperm in the ejaculate after the initial negative semen analyses has been rarely described. Often, failure of vasectomy only becomes apparent when the partner of a sterilised man becomes pregnant, and this failure has been attributed to spontaneous recanalisation of the vas deferens. Most episodes of recanalisation are believed to happen soon after vasectomy, and the pregnancy rate after vasectomy is about 1 in 2000.¹ Delayed recanalisation after the first year is uncommon, only isolated cases are reported and its true frequency is unknown. Temporary reappearance of sperm has also been reported—ie, a positive test at 12 months after vasectomy clearance is followed by two further negative samples.²

Between 1970 and 1999, over 30 000 vasectomies were done at the Elliot-Smith Clinic, Oxford, UK. The normal criteria for sterility in this clinic is two consecutive azoospermic semen samples at 16 and 18 weeks postvasectomy. Nine late failures were reported that resulted in pregnancy. In each case, the initial two postvasectomy semen samples were azoospermic, but subsequent samples at the time of pregnancy were positive. To assess more accurately the rate of recanalisation, we did a prospective study to look for reappearance of semen at 1, 2, and 3 years after initial clearance, and to see whether there was any correlation with the known pregnancy rate. Consent was obtained for patients to be contacted yearly for 3 years after initial clearance had been given. Vasectomies were done under local anaesthetic, by a group of clinic surgeons, with a standard technique. In all cases, intraluminal cautery was applied to the ends of the divided vas deferens after removal of a 1–2 cm segment. Fascial interposition and vasal ligation were not routinely undertaken, nor were clips applied. Only men in whom two consecutive samples at 16 and 18 weeks postvasectomy showed azoospermia, were included in the study.

At the time of analysis, 2250 men had been followed up for at least 1 year after vasectomy clearance. 1400 of these men had reached 2 years follow-up and 1000 had reached 3 years follow-up. Of these, 20 men had a positive semen analysis, 15 at the first year, four at the second year, and one at the third year, the rate of positive tests being significantly higher at 1 year ($p=0.02$, χ^2 test). In those men with a positive test at either the second or third year, none had had a positive test

the previous years. The sperm count, however, was less than 10 000 mL in 17 men, and semen samples of 14 were negative 1 month later (three patients did not provide follow-up samples). Also, of those that had the next yearly check all had negative samples. None of the men reported that their partners had an unwanted pregnancy. In two cases the sperm count at 1 year was greater than 100 000 mL and in one of these a subsequent follow-up test remained positive with a count of greater than 600 000 mL. The patient was advised to have a repeat vasectomy, and histological sections of the resected vas deferens showed granuloma with a multitude of small proliferating epithelial lined channels containing spermatozoa.

Previous work in our clinic has shown that transient reappearance of sperm with low counts happens in one (0.6%) in 165 men after vasectomy clearance,² which is similar to the rate we have reported here. This rate is about ten times greater than the reported pregnancy rate after vasectomy. The persistently high sperm count seen in one patient could, however, more accurately reflect the likely pregnancy rate, and does indeed match the previously reported rates. This high count was recorded at the 12 month test, suggesting that recanalisation happens soon after vasectomy. The transient appearance of sperm happens less often in the second and third year, and the rate of occurrence becomes progressively smaller.

After vasectomy, some spermatozoa could remain in the terminal vas (ampulla), which are tortuous and formed by many tiny compartments. This makes clearance of sperm unpredictable and often very slow. Numerous studies have shown discrepancies in the number of ejaculates necessary for clearance. Marwood showed that clearance is influenced not only by frequency of ejaculation, but also by age.³ Temporary reappearance of semen may therefore arise as a result of persistence of sperm in the distal vas deferens, but undetectable in previous samples. Another possible explanation for the lowered rate of positive semen tests with time is that any tiny channels formed between the two ends of vas allowing sperm through during the first year, may close over by scarring over time. This mechanism could be associated with the development of antibodies to sperm. Microchannels were seen in the one patient who underwent repeat vasectomy in this study. The prominence of granuloma in the repeat vasectomy specimens in other series enforces the association between the two.

The technique we used to analyse semen in this study did not allow distinction between motile and non-motile sperm, nor were the samples centrifuged to look for very low numbers of sperm. Previous studies have reported no pregnancies after clearance of sperm in men with low counts of non-motile sperm at initial analysis.⁴ This finding suggests that men with low counts of non-motile sperm are no more likely than persistently azoospermic patients to cause pregnancy. Reappearance of non-motile sperm after initial azoospermia was noted in 0.8% of men postvasectomy, but no pregnancies were reported after 22 months of follow up.⁵

Our study confirms that vasectomy is an extremely reliable form of contraception, but all patients should be warned that there is no guarantee that it will not fail at some point in the future.

We thank all the staff at the Elliot-Smith Clinic, Churchill Hospital, Oxford, for their help, especially P Ashwin who organised data collection, and D Crook and I Bowler, consultant microbiologists, at John Radcliffe Hospital, Oxford, who organised the semen analyses.

- 1 Philp T, Guillebaud J, Budd D. Late failure of vasectomy after two documented analyses showing azoospermic semen. *BMJ* 1984; **289**: 77–79.
- 2 O'Brien TS, Cranston D, Guillebaud J, et al. Temporary reappearance of sperm 12 months after vasectomy clearance. *Br J Urol* 1995; **76**: 371–72.

- 3 Marwood RP. Disappearance of spermatozoa from ejaculate after vasectomy. *BMJ* 1979; **1**: 87.
- 4 Davis AH, Sharp RJ, Cranston D, et al. The longterm outcome following special clearance after vasectomy. *Br J Urol* 1990; **66**: 211–12.
- 5 De Knijff DW, Vrijhof HJ, Arends J, Janknegt RA. Persistence or reappearance of nonmotile sperm after vasectomy: does it have clinical consequences? *Fertil Steril* 1997; **67**: 332–35.

The Elliot-Smith Clinic, Churchill Hospital, Oxford OX3 7DL, UK
(N Haldar FRCS, D Cranston DPhil, E Turner RGN, I MacKenzie MD,
J Guillebaud MA)

Correspondence to: D Cranston

New dopaminergic neurons in Parkinson's disease striatum

Michelle J Porritt, Peter E Batchelor, Andrew J Hughes,
Renate Kalnins, Geoffrey A Donnan, David W Howells

A new population of dopaminergic neurons has been identified in Parkinson's disease striatum. These neurons are sufficiently numerous to have an important effect on dopaminergic function in the striatum.

Parkinson's disease has been viewed as a disease caused by loss of the nigrostriatal dopaminergic projection. However, evidence is emerging from studies of parkinsonian rats and monkeys^{1,2} for the appearance of additional dopaminergic neurons within the striatum itself. We have used dopamine transporter (DAT) and tyrosine hydroxylase (TH) immunohistochemistry to look for evidence of such neurons in post-mortem tissue from the striatum of ten patients who satisfied accepted clinical and neuropathological criteria for idiopathic Parkinson's disease (age 79.6 [SD 1.5] years; post-mortem delay 32.1 [7.2] h; disease duration 11.1 [1.3] years; L-DOPA treatment duration 9.2 [1.2] years; final L-DOPA dose 806 [141] mg/day), and five age-matched controls (age 72.8 [5.6] years, post-mortem delay 12.8 [2.4] h) with no known neurological or psychiatric disease. No patient had Alzheimer's disease or dementia with Lewy bodies.

We obtained 20 µm striatal sections cut between the levels of the mamillary bodies and mamillothalamic tract from the Victorian Parkinson's disease Brain Bank which obtains informed consent before donation of tissues. To detect immunoreactive DAT (DAT⁺), rat antibody (Chemicon, Temecula, USA) directed against the N-terminus of the human dopamine transporter³ was used. To detect antibody to TH, we used a rabbit antibody (Calbiochem, San Diego, USA), which reacts with most mammalian tyrosine hydroxylases. Unlike tyrosine hydroxylase which is common to dopaminergic and noradrenergic neurons, the dopamine transporter is found only on dopaminergic neurons. Visualisation was done with a nickel-cobalt intensified immunoperoxidase procedure (Vector Laboratories, Burlingame, USA).

In the controls, the pattern of DAT immunoreactivity was essentially the same as reported previously.³ Dopaminergic

fibres were seen coursing through the internal capsules and globus pallidus providing a dense innervation of the caudate nucleus and putamen, where dopaminergic terminals were clearly visible. In addition, small numbers of DAT⁺ neurons (up to three per section) were found scattered through the putamen, caudate nucleus, globus pallidus, and internal capsule (table). In the brains of patients who had Parkinson's disease, there was loss of axon and terminal staining in the caudate nucleus and more extensive loss in the putamen with relative preservation of immunoreactivity along the boarder of the putamen and its boundary with the globus pallidus.³ However, unlike the controls, significantly increased numbers of DAT⁺ neurons were seen with an average of 26 DAT⁺ neurons per section (table). Most of these cells were small in diameter (8–15 µm) with unipolar or bipolar projections seen in a third (figure). A small subset of cells (five of 258 examined) were slightly larger (10–18 µm) with many short filamentous projections. 39.9% of the DAT⁺ neurons were found in the putamen, 11.6% in the caudate nucleus, 16.3% in the globus pallidus externa, 6.2% in the globus pallidus interna, and 25.9% in the adjacent internal capsule and in particular the ansa lenticularis. In eight of the ten brains from patients with Parkinson's disease, most (82%) DAT⁺ neurons in the putamen were located within or adjacent to the band of preserved nigral input. Tyrosine hydroxylase immunohistochemistry detected neurons with the same morphology and distribution as DAT immunohistochemistry (figure).

Because the striatum extends for about 5.1 cm (Glenda Halliday, personal communication), it is estimated that there may be as many as 66 000 dopaminergic neurons in the entire striatum and associated globus pallidus and internal capsule. This is about half the number of dopaminergic neurons that survive in the Parkinson's disease substantia nigra, equivalent to the number thought to project specifically to the putamen and approaches the 80 000 fetal mesencephalic dopaminergic neurons believed to survive and provide clinical benefit after grafting into the putamen of patients with Parkinson's disease.⁴ Thus, these neurons are sufficiently numerous to have an effect on dopaminergic function in the striatum.

The increase in number of striatal dopaminergic neurons in patients with Parkinson's disease and similar increases in the striatum of 6-hydroxydopamine treated rats² and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated parkinsonian monkeys¹ suggests that their function is to compensate for loss of nigral input to the caudate nucleus and putamen. However, the distribution would seem to contradict this argument since the new neurons are most densely packed where dopaminergic innervation from the midbrain is best preserved, in the caudate nucleus and at the pallidal boundary of the putamen. This distribution might be explained if increased dopamine turnover by the preserved nigral input, which would be enhanced by treatment with L-DOPA, caused the appearance of these striatal dopaminergic neurons. The observations that treatment of rats with amphetamine,² or L-DOPA⁵ can induce the appearance of apparently dopaminergic neurons and the concentration of these DAT⁺ neurons in regions with preserved nigral input

Position of DAT immunoreactive neurons

	Total per section	Putamen	Caudate nucleus	Internal capsule	Globus pallidus interna	Globus pallidus externa
Control (n=5)						
Mean (SEM) number	3.2 (0.4)	0.2 (0.2)	1.4 (0.5)	0.8 (0.5)	0.2 (0.2)	0.6 (0.4)
Total (%)	100	43.8	6.3	25.0	6.3	18.8
Projections present (%)	31	0	28.6	25.0	100	33.3
Parkinson's disease (n=10)						
Mean (SEM) number	25.8 (2.7)*	10.3 (1.3)†	3.0 (4.2)‡	6.7 (1.6)‡	1.6 (0.5)‡	4.2 (1.0)‡
Total (%)	100	39.9	11.6	25.9	6.2	16.3
Projections present (%)	33	31	26	41.8	43.8	33.3

*p<0.0001; †p<0.001; ‡p<0.05. Comparison between control and Parkinson's disease groups made using a t test assuming equal variances.

Number and distribution of DAT immunoreactive neurons