presence of paralytic shellfish poison in seal carcasses and in a composite mussel sample from the area. Further characterization of the virus as well as investigation of its origin are ongoing, and a rescue and rehabilitation programme for the remaining animals has been initiated by an international collaborative effort.

Albert Osterhaus, Jan Groen
Hubert Niesters
Erasmus University Hospital Rotterdam, Institute of Virology, dr. Molewaterplein 50, 3015 GE Rotterdam, The Netherlands
Marco van de Bildt, Byron Martina
Lies Vedder
Seal Rehabilitation and Research Centre, Hoofdstraat 94A, 9968 A6 Pieterburen, The Netherlands
Joseph Vos, Hans van Egmond
National Institute of Public Health and The Environment, PO Box 1, 3720 BA Bilthoven, The Netherlands
Ba Abou Sidi, Mohamed Ely Ould Barham
Centre National de Recherches Oceanographiques et des Peches, B.P. 22, Nouadhibou, Mauritania

1. Osterhaus, A. D. M. E. & Vedder, E. J. *Nature* **335,** 20 (1988).
2. Barrett, T. *et. al. Vet. Microbiol.* **44,** 261–265 (1995).
3. Osterhaus, A. D. M. E. *et. al. Vet. Rec.* **130,** 141–142 (1992).
4. Chomczynski, P. & Sacchi, N. *Anal. Biochem.* **162,** 156–159 (1987).
5. Baron, M. D. & Barrett, T. *J. Gen. Virol.* **76,** 593–602 (1995).

α-Synuclein in Lewy bodies

Lewy bodies, a defining pathological characteristic of Parkinson's disease and dementia with Lewy bodies (DLB)[1–4], constitute the second most common nerve cell pathology, after the neurofibrillary lesions of Alzheimer's disease. Their formation may cause neurodegeneration, but their biochemical composition is unknown. Neurofilaments and ubiquitin are present[5–8], but it is unclear whether they are major components of the filamentous material of the Lewy body[9,10]. Here we describe strong staining of Lewy bodies from idiopathic Parkinson's disease with antibodies for α-synuclein, a presynaptic protein of unknown function which is mutated in some familial cases of the disease[11]. α-Synuclein may be the main component of the Lewy body in Parkinson's disease. We also show staining for α-synuclein of Lewy bodies from DLB, indicating that the Lewy bodies from these two diseases may have identical compositions.

We studied formalin- or ethanol-fixed, paraffin-embedded tissue sections of substantia nigra from six patients with idiopathic Parkinson's disease, and from four patients with DLB (all clinically and neuropathologically confirmed cases), as well as cingulate cortex from the DLB patients (one with additional Alzheimer's disease pathology). We stained the tissue with affinity-purified, anti-α-synuclein serum PER2 (diluted 1:200)[12]. This antibody, raised against a synthetic peptide corresponding to residues 116–131 of human α-synuclein, specifically recognizes α-synuclein on immunoblots of human cerebral cortex extracts and does not cross-react with the related β-synuclein[12]. We also stained the sections with anti-β-synuclein serum PER3 (diluted 1:200) raised against a peptide corresponding to residues 99–111 of human β-synuclein[12]. As an absorption control we pre-incubated diluted PER2 overnight at 4 °C with 10 μM recombinant human α-synuclein. For immunohistochemistry we used avidin–biotin, with diaminobenzidine as the chromogen[6,12].

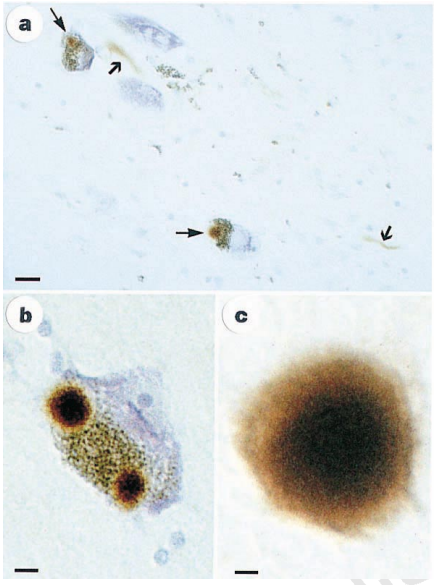

Figure 1 Substantia nigra from patients with Parkinson's disease (from the MRC Cambridge Brain Bank) immunostained for α-synuclein. **a,** Two pigmented nerve cells, each containing an α-synuclein-positive Lewy body (thin arrows). Lewy neurites (thick arrows) are also immunopositive. Scale bar, 20 μm. **b,** A pigmented nerve cell with two α-synuclein-positive Lewy bodies. Scale bar, 8 μm. **c,** α-Synuclein-positive, extracellular Lewy body. Scale bar, 4 μm.

Substantia nigra sections from Parkinson's disease and DLB, and cingulate cortex sections from DLB and DLB with Alzheimer's disease incubated with the α-synuclein antibody PER2 showed staining of numerous brainstem-type and cortical Lewy bodies (Figs 1 and 2). Lewy neurites, which are dystrophic processes with the same immunohistochemical staining profile as Lewy bodies, were also reactive (Figs 1a, 2a). The strong staining made it difficult to distinguish between the core and the corona of the brainstem-type Lewy bodies (Figs 1, 2b). The staining was specific, and did not occur after pre-adsorption of the primary antibody with recombinant α-synuclein.

We obtained similar results with antibody PER1, raised against a synthetic peptide corresponding to residues 11–34 of human α-synuclein, indicating that full-length α-synuclein may be present in the Lewy body (data not shown). We found no specific staining of Lewy bodies or Lewy neurites with the β-synuclein antibody PER3.

We also stained tissue sections from Parkinson's disease and DLB with the ubiquitin monoclonal antibody 1510 (Chemicon, diluted 1:500)[13]. Double-staining of substantia nigra sections from Parkinson's disease with PER2 and 1510, showed staining of similar numbers of Lewy bodies and neurites with each antibody. Similarly, in adjacent tissue sections of substantia nigra and cingulate cortex from DLB, we found comparable numbers of Lewy bodies and neurites stained with PER2, antibody 1510 or undiluted neurofilament antibody RMO32, a monoclonal antibody to a phosphorylated epitope in the mid-sized neurofilament subunit which

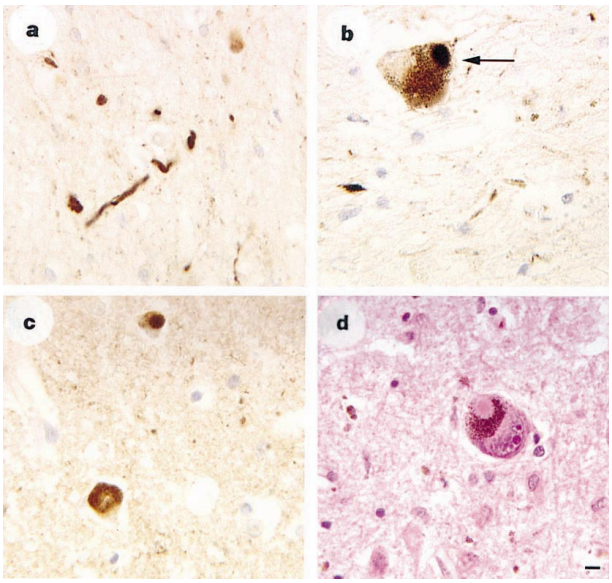

Figure 2 Tissue from patients with DLB (from the tissue collection of the Department of Pathology and Laboratory Medicine, University of Pennsylvania) immunostained for α-synuclein. **a,** α-Synuclein-positive Lewy neurites in the substantia nigra. **b,** α-Synuclein-positive Lewy body (arrow) in pigmented nerve cell of the substantia nigra. **c,** Two α-synuclein-positive Lewy bodies in the cingulate cortex. **d,** Haematoxylin and eosin-stained section of substantia nigra with a pigmented nerve cell containing a Lewy body. Scale bar for **a**–**d**, 10 μm.

specifically recognizes Lewy bodies[6]. Cortical Lewy bodies immunoreactive to PER2 had a similar morphology to the ubiquitin- and neurofilament-positive Lewy bodies in that they had round, oval or irregular shapes and frequently displaced the nucleus to one side (Fig. 2c).

The strong α-synuclein staining of brainstem-type and cortical Lewy bodies in idiopathic Parkinson's disease and DLB shows that α-synuclein is a component of the Lewy body. In some familial cases of Parkinson's disease there is an alanine to threonine mutation at residue 53 of α-synuclein[11]. A major effect of this mutation may be to promote the aggregation of α-synuclein into filaments, resulting in the formation of Lewy bodies. The Parkinson's disease cases that we studied were non-familial, so at least two distinct pathogenetic mechanisms can lead to α-synuclein aggregation. α-Synuclein aggregation and Lewy-body formation may be important in the aetiology and pathogenesis of all cases of Parkinson's disease.

As brainstem-type and cortical Lewy bodies from DLB were strongly immunoreactive for α-synuclein, α-synuclein aggregation may also underlie Lewy-body formation in this condition. The intracytoplasmic Lewy body is therefore central to the neurodegenerative process, and both Parkinson's disease and DLB may be α-synuclein diseases.

Maria Grazia Spillantini
Medical Research Council Centre for Brain Repair and Department of Neurology, University of Cambridge, Robinson Way, Cambridge CB2 2PY, UK
Marie Luise Schmidt
Virginia M.-Y. Lee
John Q. Trojanowski
Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-4283, USA
Ross Jakes, Michel Goedert
Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

1. Lewy, F. H. in *Handbuch der Neurologie* Band III (ed. Lewandowski, M.) 920–933 (Springer, Berlin, 1912).
2. Trétiakoff, M. C. Thesis, Univ. Paris (1919).
3. Okazaki, H., Lipkin, L. E. & Aronson, S. M. *J. Neuropathol. Exp. Neurol.* **20,** 237–244 (1961).
4. Kosaka, K., Oyanagi, S., Matsushita, M., Hori, A. & Iwase, S. *Acta Neuropathol.* **36,** 221–233 (1976).
5. Goldman, J. E., Yen, S.-H., Chiu, F.-C. & Peress, N. S. *Science* **221,** 1082–1084 (1983).
6. Schmidt, M. L. *et al. Am. J. Pathol.* **139,** 53–65 (1991).
7. Kuzuhara, S., Mori, H., Izumiyama, N., Yoshimura, M. & Ihara, Y. *Acta Neuropathol.* **75,** 345–353 (1988).
8. Lennox, G., Lowe, J., Morrell, K., Landon, M. & Mayer, R. J. *J. Neurol. Neurosurg. Psychiat.* **52,** 67–71 (1989).
9. Duffy, P. E. & Tennyson, V. M. *J. Neuropathol. Exp. Neurol.* **24,** 398–414 (1965).
10. Roy, S. & Wolman, L. *J. Pathol.* **99,** 39–44 (1969).
11. Polymeropoulos, R. *et al. Science* **276,** 2045–2047 (1997).
12. Jakes, R., Spillantini, M. G. & Goedert, M. *FEBS Lett.* **345,** 27–32 (1994).
13. Shaw, G. & Chau, V. *Proc. Natl Acad. Sci. USA* **85,** 2854–2858 (1988).

Endogenous proviruses as "mementos"?

In a recent News and Views[1], J. P. Stoye discussed the potential health risks associated with the spread of endogenous proviruses from pigs and primates through the use of their organs for transplantation into humans. Although the risks to patients and the public from horizontal transmission may be as manageable as he represents, we would like to comment on his reference to the presence of vertically transmitted proviruses in the genomes of all mammals as "mementos".

The spread of such parasites throughout the genome of a species is expected to be accompanied by a large number of additional 'random' insertions. Many (perhaps most) of these insertions will be associated with mutations that are ultimately eliminated by natural selection, so the "mementos" are the surviving insertions, those with minimal effects on the host's fitness. For example, laboratory experiments show that the recent and rapid spread of the *P* element throughout the genomes of all *Drosophila melanogaster* in nature was accompanied by enormous transient net reductions in fertility and viability in the population[2,3]. But in a few thousand *Drosophila* generations, there will be only "mementos" of the *P* element in the genomes of *D. melanogaster* (much as in seen in other species of *Drosophila*).

The apparently innocuous presence of these parasites does not mean that they have not caused a great toll in the past. Further, there is evidence that host genomes may have evolved the ability to repress rapid transposition of families of retroviruses[4] or retrotransposable elements[5], so that mutation of the relevant genes leads to element mobilization. There is therefore a risk that xenotransplantation could result in such mobilization in a foreign genetic background that lacks appropriate genes to repress transposition. Indeed, Stoye notes that mammalian hosts have evolved "adaptations of host viral-receptor proteins" that contain the spread of the endogenous proviruses. The ubiquitous presence of such effective adaptations implies a strong selective force (differential viability and/or fertility) on the hosts in the past.

In the absence of effective control of reproduction, there is a real risk that a germline infection in a xenotransplantation patient could introduce a new and potentially virulently mutagenic endogenous provirus into the human gene pool. Although the risks associated with insertional mutations are distributed over future generations, they are nevertheless great and worthy of serious consideration in the evaluation of risks and benefits inherent to an expansion of xenotransplantation.

Charles H. Langley
Center for Population Biology, University of California, Davis, California 95616, USA
Brian Charlesworth
Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh EH9 3JT, UK

Stoye replies — On the basis of analogies between *P* elements and retroviruses, Langley and Charlesworth suggest that one potential risk associated with xenotransplantation is a form of insertional mutagenesis resulting from germline integrations by retroviruses derived from endogenous proviruses in the engrafted organs. I agree that these elements are potentially hazardous, but I am not convinced that the threat posed by this form of genomic bombardment is great enough to figure significantly in risk–benefit analysis of xenotransplantation. Rather, the much greater risk is that posed by these elements acting as infectious agents of disease.

Experiments in mice show that exogenous infection by retroviruses[6], or activation of endogenous proviruses[7], can result in germline infection. However, even under the most favourable circumstances, the number of acquired proviruses seems to be less than one per generation, of which at most one in ten is likely to be mutagenic[8,9]. Increases in the number of germline proviruses will require further cycles of maternal expression followed by oocyte infection[10], a process predicted to be significantly less efficient than *P*-element mobilization associated with hybrid dysgenesis in *Drosophila*.

To pose any significant risk to human fitness, a very high frequency of horizontal retroviral transmission (followed by infection of the germ line) would be required. For horizontal transmission on such a scale to occur, levels of viraemia associated with unacceptably high probabilities of virally induced disease would almost certainly have to occur. Several groups are currently addressing the question of whether xenotransplantation procedures pose any threat of infectious disease; provided that this concern is met, the threat to the human gene pool seems exceedingly remote.

Jonathan Stoye
National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK
e-mail: jstoye@nimr.mrc.ac.uk

1. Stoye, J. P. *Nature* **386,** 126–127 (1997).
2. Kidwell, M. G., Kimura, K. & Black, D. M. *Genetics* **86,** 815–828 (1988).
3. Engels, W. R. *Genetics* **145,** 11–15 (1997).
4. Best, S., Le Tissier, P., Towers, G. & Stoye, J. P. *Nature* **382,** 826–829 (1996).
5. Pelisson, A. *et al. EMBO J.* **13,** 4401–4411 (1994).
6. Panthier, J. J., Condamine, H. & Jacob, F. *Proc. Natl Acad. Sci. USA* **85,** 1156–1160 (1988).
7. Rowe, W. P. & Kozak, C. A. *Proc. Natl Acad. Sci. USA* **77,** 4871–4874 (1980).
8. Jenkins, N. A. & Copeland, N. G. *Cell* **43,** 811–819 (1985).
9. Lock, L. F., Jenkins, N. A. & Copeland, N. G. *Curr. Top. Microbiol. Immunol.* **171,** 27–41 (1991).
10. Lock, L. F. *et al. EMBO J.* **7,** 4169–4177 (1988).