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The human prion diseases: from neuropathology to pathobiology and molecular genetics

Final report of an EU Concerted Action

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1 Introduction

This European Union BIOMED-1 Concerted Action (CA) was proposed in May 1992. Although it subsequently made the Short List of projects to be funded, the contract was delayed for political reasons (impending integration of Austria and other nations into the EU) until 1994, with funding shared with the Austrian Federal Ministry for Science and Transport (actual receipt of funds started in December 1994). In those years, interest in the topic was limited, despite the catastrophic scale of the bovine spongiform encephalopathy (BSE) epidemic in the UK.

Work within this CA was well under way when, on 20 March 1996, the British Minister of Health announced the emergence of a new variant of Creutzfeldt-Jakob disease (CJD) in the UK that possibly had originated from the BSE epidemic. This announcement caused world-wide concern and uncertainty about the safety of beef and bovine products. In this new situation, fortunately, the present CA proved to be an ideal tool to address some important issues. We have adapted to the new situation by initiating a Europe-wide survey of the range of neuropathological CJD phenotypes and organized meetings covering respective topics in Vienna, May 31–June 1, 1996 [7]; and December 6–7, 1996 [11]. The data presented there failed to identify cases of the new CJD variant other than those reported from the UK and France. In addition, many participants of this CA had to adapt to the new situation by giving expert opinion at numerous national and international meetings and committees, including the EU and the WHO, and by providing much needed scientific information to public media. Thus information at all levels has emerged as an important by-product of this CA.

†See appendix.

2 Results

2.1 Objectives of the project (as outlined in the Work Programme)

- Establishment of the European Neuropathological Data Base on Prion Diseases (ENDAPRID).
- Establishment of the European Neuropathological Tissue Bank on Prion Diseases (ENTIPRID).
- Definition of neuropathological diagnostic criteria for prion diseases, including standardized immunocytochemistry for the prion protein (PrP).
- Definition of a safe and practicable standardized tissue handling protocol for prion diseases.
- Establishment of the Neuropathological Clearing House for Prion Diseases (ENCLEAPRID).
- Co-ordination of clinico-neuropathological investigations, molecular biological investigations, infectivity and in vitro studies, and support of local research subprojects in the field by sharing of materials and special techniques.
- Exchange of scientific and laboratory personnel on a Europe-wide scale.

2.2 Scientific and technical progress made

Originally proposed timetable

The originally proposed timetable was met.

After the preparatory phase of 3 months used for individual information from all participants and preparation

of their specific tasks according to the original timetable, all parts of this CA started. The Administrative Office of the Concerted Action (CA), of special importance because of the large number of participating groups, was established in Vienna with Mrs Heike Tagger and, later, Mrs Monika Richter as Project Secretary.

- Establishment of the *European Neuropathological Data Base on Prion Diseases* (ENDAPRID). A Case Registration Sheet was successfully worked out within the proposed timetable, to register cases for ENDAPRID.
- The tissue handling protocol was defined within the proposed timetable as a Consensus Report [4].
- Neuropathological diagnostic criteria were defined within the proposed timetable as a Consensus Report [3].
- Standardization of PrP immunocytochemistry was prepared within the proposed timetable, including a ring trial among participating laboratories, but additional experiments became necessary and have been recently completed [18].
- A progress report meeting was held on October 16, 1995, in Vienna. The meeting report was published in *Neuropathology and Applied Neurobiology* [6].
- After announcement of a possible link between BSE and nv-CJD, a meeting on 'Phenotypic variation in Creutzfeldt-Jakob disease' was held in Vienna, May 31 and June 1, 1996. The meeting report was published in *Brain Pathology* [7].
- A final progress report meeting was held December 6–7, 1996, in Vienna. The meeting report was published in *Neuropathology and Applied Neurobiology* [11].

Objectives set out in the technical annex to the contract and their implementation

These were implemented in this CA as follows:

- Establishment of the *European Neuropathological Data Base on Prion Diseases* (ENDAPRID). A Case Registration Sheet was worked out to register cases according to:

  (i) clinical diagnostic criteria as possible or probable sporadic (according to the Masters criteria used in the CA on Surveillance of CJD in the EU), accidentally transmitted or familial Creutzfeldt-Jakob disease [3] or other human prion diseases;

  (ii) neuropathological diagnostic criteria as definite human prion disease [3];

  (iii) neuropathological material available for tissue studies.

These Case Registration Sheets were sent to all CA participants and were used to register 272 retrospective and prospective cases by December 31, 1996. This number does not include most cases collected in neuropathological laboratories of established CJD Reference Centres (UK, Germany, France, Italy, Switzerland). Data from the latter national registries are accessible through collaboration with the respective CA participants.

- Establishment of the *European Neuropathological Tissue Bank of Prion Diseases* (ENTIPRID). The Case Registration Sheet includes information on material available for ENTIPRID. Material has been collected in various centres, including frozen tissue in addition to paraffin embedded tissues, and distributed on a limited scale for additional methodologies (Western blotting, PRNP genotyping).
- Definition of *neuropathological diagnostic criteria for prion diseases*. This has been fully achieved in a CONSENSUS REPORT published in *Brain Pathology* [3] and successfully applied to ENDAPRID cases.
- Standardization of immunocytochemistry for the prion protein (PrP) was started by completing a ring trial among participating laboratories. Results were presented and discussed at the CA Meeting on October 16, 1995, in Vienna, but required additional experiments that have been recently completed [18].
- A *tissue handling protocol for prion diseases* that is both safe and practicable was defined, published as a Consensus Report in *Brain Pathology* [4], and has been successfully used. It provided most useful and much needed information, especially for pathologists. Many of them have been very reluctant to perform autopsies on patients dying with suspected CJD. We hear from some that this report has greatly clarified many of the issues at stake. In addition to the original report in English, we published a version in German [5] and are encouraging translations into other languages.
- The *Neuropathological Clearing House for Prion Diseases (ENCLEAPRID)* for special evaluation of difficult, atypical or insufficiently diagnosed cases, including application of immunolabelling for the prion protein (PrP)

(immunocytochemistry and Western blotting) and genotyping of the PrP gene, was established. Diagnostic neuropathology support was provided to numerous laboratories with limited experience in these rare diseases or lack of appropriate methodology.

- *Clinico-neuropathological investigations* into the relationships between clinical phenotype and the distribution of pathology, including ultrastructural investigations and the pattern of PrP deposition and of synaptic pathology, were performed in several participating laboratories. Some insight into the mechanism of neuronal degeneration was given by the study of apoptosis/DNA fragmentation as an important mechanism for nerve cell loss in prion disease [10]. Detailed assessment of CJD cases in Austria, all confirmed by neuropathology with immunocytochemistry using antibodies made available through this CA, revealed a significant increase of diagnosed cases in recent years; this is interpreted as being due to increasing awareness among physicians and the high autopsy rate in Austria [14, 15]. Surveillance of CJD in the UK succeeded in identifying a recent temporal cluster of CJD cases with atypical clinicopathological features (new variant CJD/nv-CJD) in which origin from exposure to the BSE agent has to be considered a possibility [20]. Another nv-CJD case has been reported from France [8]. At past Vienna CA Meetings, however, a survey of neuropathological cases in most other European countries failed to identify additional cases of nv-CJD [7, 11]. Nv-CJD has been frequently equated with Kuru, the only non-iatrogenic human prion disease of confirmed infectious origin. Detailed neuropathological examination of what appears to be the last available archival set of a Kuru brain did not identify features characteristic of nv-CJD [17].
- *Molecular biological investigations* of familial and iatrogenic human prion diseases with differing phenotypes, examining mutations and polymorphisms (especially at codon 129) of *PRNP*, the gene encoding for PrP, had already a significant result. Collaboration within this CA on Gerstmann-Sträussler-Scheinker disease (GSS) in a family with both the classical ataxic and a CJD-like phenotype resulted in the new and exciting finding that, contrary to other human prion diseases, phenotypic heterogeneity of GSS is independent from the genotype at *PRNP* codon 129 [13]. This means that other factors (yet unknown genotype? infectious agent 'strain'? environmental?) are responsible for phenotypic expression and should be specifically investigated. Moreover, we were able to recently identify a family with the *PRNP* 200 mutation and homozygosity for valine at codon 129; this constellation has not been observed previously [16]. Moreover, a recently discovered family with fatal familial insomnia (FFI) [1] is currently under detailed investigation, especially with regard to correlation between genotype, tissue pathology and deposition of PrPres [12]. With regard to nv-CJD, patients have so far only met/met at *PRNP* codon 129 [20].
- Improvement of neuropathological experience and application of modern diagnostic and research techniques in prion diseases was fostered by Europe-wide *exchange of scientific/laboratory personnel*, although on a scale smaller than originally anticipated.

2.3 ENDAPRID results

In addition to results given above, a detailed analysis was carried out on cases submitted to ENDAPRID. By December 31, 1996, 272 cases had been submitted.

Country of origin (Fig. 1)
The 272 ENDAPRID cases do not include most of the recent cases diagnosed in large countries with established CJD reference centres such as the UK, Germany, France and Italy. Data from these are available by direct contact with the respective reference centre. Most ENDAPRID cases were submitted from Austria; these Austrian cases include all retrospective cases as well. One laboratory in Germany, one in Italy, one in Slovenia, and Norway, Finland and Denmark submitted also retrospective cases. Other submitters sent mainly a few newly diagnosed cases for diagnostic confirmation.

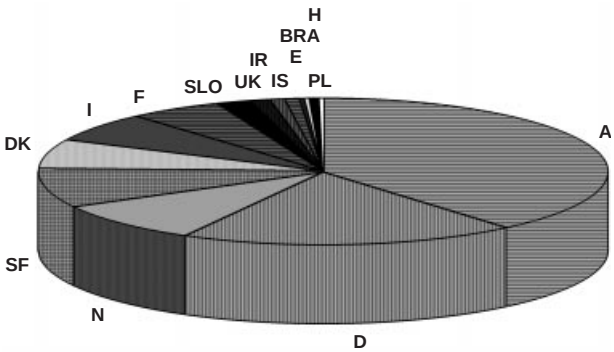

Figure 1. Two hundred and seventy-two ENDAPRID cases: country of origin.

We acknowledge the contribution of the 29 ENDAPRID submitters from 13 countries: Bergmann, Bremen, Germany (D); Bojsen-Mœller, Arhus, Denmark (DK); Budka, Vienna, Austria (A); Cabello, Madrid, Spain (E); Deslisle, Toulouse, France (F); Georgsson, Reykjavik, Iceland (IS); Gray, Paris, France (F); Gullotta, Münster, D; Haltia, Helsinki, Finland (SF); Ironside, Edinburgh, UK; Jellinger, Vienna, A; Keohane, Cork, Ireland (IR); Kleinert, Graz, A; Kopp, Lyon, F; Laursen, Copenhagen, DK; Liberski, Lodz, Poland (PL); Maier, Innsbruck, A; Mikol, Paris, F; Montagna, Rio de Janeiro, Brazil (BRA); Pilz, Salzburg, A; Popovic, Ljubljana, Slovakia (SLO); Scaravilli, London, UK; Schmidbauer, Oberwart, A; Schröder, Aachen, D; Skullerud, Oslo, Norway (N); Stering, Eisenerz, A; Syré, Linz, A; Teglbjaerg, Aalborg, DK; Trabattoni, Parma, Italy (I).

Year of death of ENDAPRID cases (Fig. 2)

According to the development of this CA, most cases were submitted from 1995; submission from 1996 by December 31 is still incomplete. However, there is also a significant proportion of retrospective cases from earlier years, although from the 1970s and early 1980s it is rather fragmentary.

Neuropathological categorization (Fig. 3)

Of the total of 272 cases, 232 cases were neuropathologically diagnosed as definite CJD by histopathology including immunocytochemical demonstration of PrPres [3]. Except for earlier reported cases of nv-CJD from the UK and France [8, 20], none of the other cases had histopathological and PrP immunocytochemical features of nv-CJD. One case from Austria had a dura mater graft to the frontal region 11 years before death from CJD. No other risk for iatrogenic transmission was reported; as mentioned, most cases from France and the UK where most iatrogenic cases have been observed [2] are primarily registered with the respective national reference centres. Three recent Austrian cases were diagnosed, by pathology and molecular genetics, as FFI [1]. There were no other evident familial cases reported, although for the large majority of investigated cases PRNP genotyping was not available.

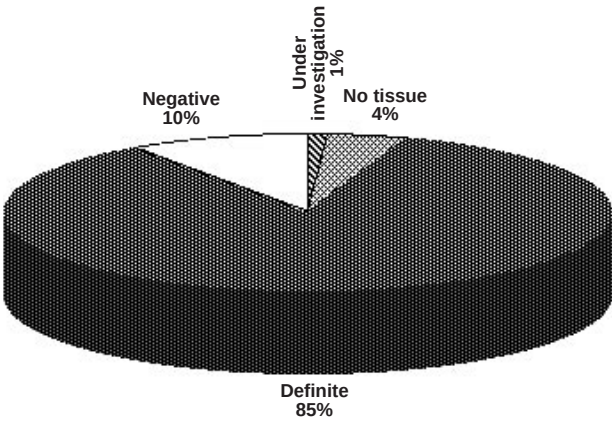

Figure 3. ENDAPRID: neuropathological categorization (272 cases).

Clinico-pathological correlation (Fig. 4)

Clinical data were derived from the Case Submission Sheet filled by submitting neuropathological laboratories and categorized as probable or possible CJD according to the modified Masters criteria [4] currently used in the CA on Surveillance of CJD in the EU (Project Leader: Dr R. G. Will, Edinburgh). Although such summary data must be less exact than detailed data from a prospective clinical survey and need to be interpreted with caution, they deserve some consideration. In this series, only half of the

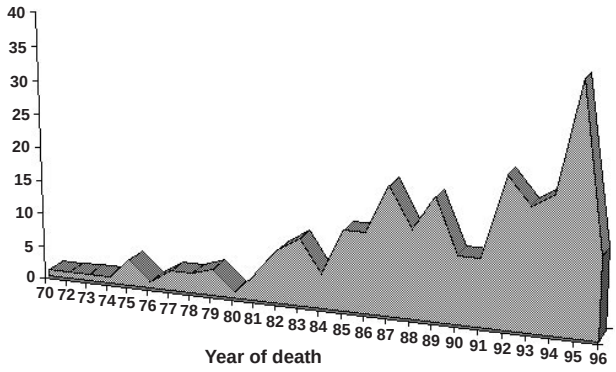

Figure 2. Two hundred and seventy-two ENDAPRID cases per year of death.

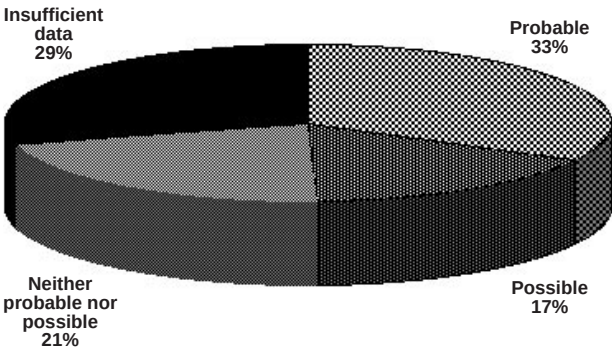

Figure 4. Definite CJD (232 cases): clinical correlation.

definite CJD cases had reported criteria of probable or possible CJD. When only the 164 cases with sufficient data were considered, 46% were clinically probable CJD, 24% clinically possible, and 30% neither. Thus there is in this large neuropathological series a significant proportion of cases that did not have reported clinical criteria of probable or possible CJD. While some of this might result from inadequate reporting to, and by, referring neuropathologists, it is interesting to note that a similar result of a significant proportion of clinically atypical cases was found in a retrospective neuropathological study of definite CJD from Austria; there 17% of cases, after careful retrospective analysis of case histories, did not fulfil criteria of probable or CJD either [14, 15]. One possible conclusion might be that current clinical criteria for CJD, by recognizing mainly classical presentation, have only moderate sensitivity. This important issue should be evaluated by further prospective detailed clinico-neuropathological correlation. What has become clear, however, is that only a broad-based neuropathological examination of possible suspects, including less typical neurodegenerative conditions, will identify a maximum of human TSE cases.

With regard to the specificity of clinical diagnostic criteria, it is reassuring that most neuropathologically negative cases were clinically categorized as neither probable nor possible cases. However, two cases with clinical criteria of probable CJD turned out neuropathologically to be Alzheimer's disease (Fig. 5).

2.4 Major achievements arising directly from the project

The definition both of standardized neuropathological diagnostic criteria [3] and tissue handling [4] in human prion diseases can be considered as important achievements. They enable diagnosis and handling in these rare diseases according to uniform criteria that is of utmost importance for meaningful and co-ordinated multi-centre research. Moreover, they will serve as important reference bases for eventual revisions to include also the new CJD variant (nv-CJD) in which an origin from BSE has now become probable. Standardization of immunocytochemistry for PrP rapidly becomes most important for appropriate diagnosis of definite human prion diseases [18]. Assessment of the phenotypic range of CJD in Europe is equally important, especially with regard to emergence of nv-CJD. The survey of the neuropathological features of CJD in Europe by this CA was an important first step, suggesting confinement of nv-CJD to the UK and France at present, but needs to be followed up in the future. The data on transmissibility of BSE to non-human primates [19] and the characteristic PrP 'signature' on Western blots of various prion diseases [9] have become essential scientific evidence in the ongoing discussion about the probability of BSE spread to humans.

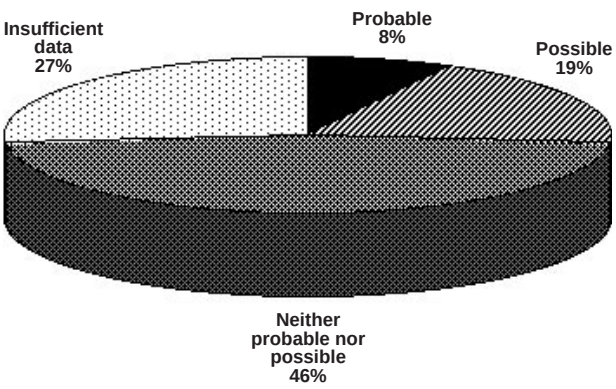

Figure 5. Neuropathology negative for CJD in 26 cases: clinical correlation

3 General information on the project

3.1 Safety considerations

The published Consensus Report on tissue handling in human prion diseases [4] is of major importance for safety considerations in dealing with these transmissible diseases. The risk for medical personnel, including personnel performing autopsies, is specifically addressed and relevant precautions and safety measures are proposed.

3.2 Benefits of the project and its community added value

It is very difficult to imagine that major achievements of this project, namely the

- consensus definition of diagnostic criteria for human prion diseases;
- consensus definition of appropriate tissue handling;
- standardization of PrP immunocytochemistry, and
- preliminary assessment of the phenotypic range of CJD in Europe, especially with regard to nv-CJD, as well as
- networking of European neuropathological laboratories as basis of this CA, with collection of data and materials,

would have been reached without this large-scaled European collaboration.

3.3 Difficulties encountered at scientific and/or management level

The return rate of case registration for ENDAPRID initially has been less than originally expected. This might reflect the fact that for some groups, the retrieval work necessary for this task requires more time than anticipated in the original timetable. The consequence is that frequent reminders are mandatory to ensure compliance is as complete as possible with all aims of a CA on as large a scale as this.

Some difficulties arose from the fact that the scientific community, including most participants of this CA, were originally informed about the new situation caused by nv-CJD not via scientific or CA pathways, but via the mass media. Thus a period of uncertainty ensued, being finally clarified by the full scientific publications. This episode emphasizes the need for improved and uninhibited information flow between scientists, even when highly sensitive and politicized areas and data requiring confidentiality are involved.

3.4 Plans for the future

This CA ended by December 31, 1996. However, the new situation created by the emergence of nv-CJD urgently required uninterrupted continuation of this effort. Serving this urgent need, we submitted in June 1996 a similar and expanded CA proposal 'Human Transmissible Spongiform Encephalopathies: Neuropathology and Phenotypic Variation' to the EU BIOMED-2 programme. The proposal received high marks at reviewing and was put on the Short List. Contracts were signed in May 1997, and the new CA started officially on 1 July 1997.

3.5 Acknowledgements

Since the EU financial contribution to this CA was cut to ECU 50,000, execution of this programme would not have been possible without very significant financial support from the Austrian Government (Bundesministerium für Wissenschaft, Forschung und Kunst) which was funding no less than 77.78% of the total CA budget of ECU 225,000. This project would not have been successful without help and support from many persons. Specifically, the project leader has to thank the numerous European neuropathologists who contributed to make this effort truly European and motivated him to carry on with a new Concerted Action. Finally, the staff in Vienna were indispensable for the work of this CA: Dr J. A. Hainfellner who served as Project Assistant, Drs Ch. Jarius-Fuhrmann, C. Radbauer, and J. Wanschitz, who worked for some time as Project Neuropathologists, Mrs H. Flicker, who as Project Head Technician contributed immensely to optimization of PrP immunocytochemistry, and last but not least Mrs H. Tagger and M. Richter who as Project Secretaries were at the very heart of this effort.

Appendix

Participants of the Concerted Action

EU countries

Austria: Prof. H. Budka and Dr J. A. Hainfellner, Vienna; Prof. K. Jellinger, Vienna; Doz. R. Kleinert, Graz; Dr H. Maier, Innsbruck; Dr P. Pilz, Salzburg. *Belgium:* Dr R. Sciot, Leuven; Prof. J. J. Martin, Antwerpen. *Denmark:* Dr M. Bojsen-Moller, Arhus; Dr H. Laursen, Copenhagen; Dr P. St Teglbjaerg, Aalborg. *Finland:* Prof. M. Haltia, Helsinki; Dr J. Kovanen, Helsinki. *France:* Prof. M.-B. Delisle, Toulouse; Prof. D. Dormont and Dr C. Lasmézas, Fontenay aux Roses; Prof. F. Gray, Garches and Creteil; Prof. J.-J. Hauw and Dr D. Seilhean, Paris; Dr N. Heldt, Strasbourg; Prof. J. Mikol, Paris; Prof. C. Vital, Bordeaux; Prof. J.-F. Foncin and Dr K. El Hachimi, Paris; Prof. N. Kopp, Lyon. *Germany:* PD M. Bergmann, Bremen; Prof. T. Bilzer, Düsseldorf; Prof. J. Cervos-Navarro, Berlin; Prof. H. Diringer, Berlin; Prof. W. Feiden, Homburg/Saar; Prof. H. H. Goebel and Dr J. Bohl, Mainz; Prof. F. Gullotta, Münster; PD K. Jendroska, Berlin; Prof. H. A. Kretzschmar, Göttingen; Prof. R. P. Linke, Martinsried b. München; Prof. R. Meyermann and PD J. W. Boellaard, Tübingen; Prof. P. Mehraein, München; Prof. W. Schlote, Frankfurt/Main; Prof. J. M. Schröder, Aachen; Prof. R. Schröder, Köln; Prof. B. Volk, Freiburg i. Br; Prof. G. F. Walter and PD A. Hori, Hannover; Prof. O. D. Wiestler, Bonn. *Greece:* Prof. St J. Balloyannis, Thessaloniki; Prof. P. Davaki and Prof. E. Patsouris, Athens. *Iceland:* Prof. G. Georgsson, Reykjavik. *Ireland:* Dr. M. Farrell, Dublin; Dr C. Keohane, Cork. *Italy:* Prof. O. Bugiani, Milano; Prof. G. Macchi and Dr C. Masullo, Roma; Prof. G. Costanzi, Milano; Prof. M. Pocchiari, Roma; Prof. N. Rizzutto, Verona; Prof. D. Schiffer, Torino; Dr G. R. Trabattoni, Parma; Prof. F. Giangaspero, Cesena. *Netherlands:* Dr G. H. Jansen, Utrecht. *Norway:* Prof. B. Grinde, Oslo; Prof. S. J.

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Non-EU countries

Hungary: Dr K. Majtenyi, Budapest; *Poland:* Prof. P. P. Liberski, Lodz. *Romania:* Dr A. Petrescu, Bucharest. *Slovakia:* Dr E. Mitrova, Bratislava and Budapest. *Slovenia:* Dr M. Popovic, Ljubljana. *Switzerland:* Prof. A. Aguzzi, Zürich; Prof. R. C. Janzer, Lausanne; Dr G. Pizzolato, Geneva; Prof. B. Stamm, Aarau; Prof. M. Vandevelde, Bern.

Observers

Australia: Prof. C. L. Masters, Melbourne. *Japan:* Prof. J. Tateishi, Fukuoka; Prof. T. Kitamoto, Sendai. *USA:* Dr P. Brown, Bethesda.

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