Clin. exp. Immunol. (1988) **71**, 229–234

Rapid diagnosis of rabies and post-vaccinal encephalitides

M. J. WARRELL, S. LOOAREESUWAN, S. MANATSATHIT, N. J. WHITE, P. PHUAPRADIT, A. VEJJAJIVA, C. H. HOKE, D. S. BURKE & D. A. WARRELL *Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok; Sir William Dunn School of Pathology and Nuffield Department of Clinical Medicine, University of Oxford, UK; Bamrasnaradura Hospital for Infectious Diseases; Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University and Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand*

(Accepted for publication 30 September 1987)

SUMMARY

In an attempt to establish the diagnoses of rabies post-vaccinal encephalitis (PVE) and early rabies encephalitis, paired serum and CSF levels of rabies neutralizing antibody (Rab) and rabies specific-IgM (RIgM) were compared in 12 PVE, 10 rabies and five control patients with similar presenting clinical features. Rapid methods of rabies antigen detection were evaluated in 17 patients.

All 12 PVE patients had Rab in their serum and in eight it was also present in the CSF. These same eight had RIgM in the serum, and in seven also in the CSF. The CSF antibodies may have originated in the plasma since six patients had a high albumin quotient indicating leakage across the blood–brain barrier. Among the rabies patients, only the two vaccinated ones had serum Rab; this was also detected in the CSF of one and RIgM was in the CSF of the other.

A raised IgG Index, indicating intrathecal synthesis of IgG was seen in five of 12 PVE patients. This did not correlate with the presence of CSF rabies antibody, suggesting production of antibody to other vaccine antigens of neural origin.

The diagnosis of rabies encephalitis in life was made by antigen detection in a skin biopsy. No false positive results occurred and the method was as efficient as immunofluorescence of a post-mortem brain biopsy.

Keywords rabies diagnosis CSF antibody post-vaccinal encephalitis

INTRODUCTION

Rabies and post-vaccinal encephalitis (PVE) can present indistinguishable clinical pictures. It is notoriously difficult to confirm the diagnosis of rabies encephalitis during life (Hattwick & Gregg, 1975, Anderson *et al.*, 1984), and PVE is diagnosed by exclusion. Confusion between the two may end in tragedy. Rabies vaccine of nervous tissue origin is still used in over 90% of post-exposure vaccine courses (Roumiantzeff *et al.*, 1985). Neurological reactions to Semple vaccine have recently been found in 1:120 vaccinees (Swaddiwuthipong *et al.*, 1988).

Differentiation of the immune response to rabies vaccine, and its accompanying PVE, from rabies encephalitis has been attempted in animal experiments. Rabies vaccination of dogs does not induce CSF antibody (Arko, Schneider & Baer, 1973). Studies of PVE in cavies showed no increased permeability of the blood–brain barrier to rabies antibodies, and no evidence of intrathecal antibody production (Bell & Moore, 1979). By contrast in animal rabies infection, high titres of antibody were induced in both serum and brain, with the inference that a serum:CSF antibody ratio of $< 10:1$ was evidence of recovery from rabies. Human rabies has been diagnosed on this basis in the absence of virus or antigen detection (Hattwick *et al.*, 1972, Porras *et al.*, 1976, Tillotson, 1977), but comparison of the distribution of rabies antibody in PVE and rabies has not been reported in man.

In rabies patients who have not been vaccinated, antibody usually appears late and in low titre (Hattwick & Gregg, 1975). Atanasiu, Savy & Gilbert (1978) showed that rabies-specific IgM appeared in infected patients' serum earlier than IgG or total neutralizing antibody and therefore detection of rabies-specific IgM in the CSF may be a rapid method of early diagnosis, as has been used for other viral encephalitides, notably Japanese encephalitis (Burke *et al.*, 1985). From the cavy PVE model, rabies specific-IgM would not be expected in the CSF of patients with PVE.

In this study serum and CSF levels of total rabies neutralizing antibody (Rab) and of IgM class rabies antibodies were compared in patients with PVE and rabies encephalitis, with and without rabies vaccination. The function of the blood–brain barrier was investigated by calculation of the albumin quotient (Q_{alb}) and evidence of intrathecal IgG synthesis was sought by calculation of the IgG index. In a smaller group of patients,

Correspondence: Dr M. J. Warrell, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE, UK.

rapid methods of diagnosing rabies encephalitis were evaluated. The ethical committee of the Faculty of Tropical Medicine, Mahidol University approved the study.

PATIENTS

Twelve patients with post-vaccinal encephalitis (PVE), ten with rabies encephalitis and five 'control' patients presenting with similar symptoms were admitted to hospitals in Bangkok. The diagnosis of PVE was made clinically by exclusion of other likely diseases in recent recipients of rabies vaccine. Rabies infection was confirmed by at least one of the following methods: immunofluorescent (IF) detection of antigen in brain smears (Dean & Abelseth, 1973); in a skin biopsy (Bryceson *et al.*, 1975) (see below), or by isolation of virus.

MATERIALS AND METHODS

Paired serum and lumbar cerebrospinal fluid (CSF) samples were taken on up to three occasions from each patient and were stored in aliquots at −70°C. To prevent error from mechanical contamination from the blood, a cell count was made on the fresh CSF and any sample with more than 80 erythrocytes per μl was excluded. CSF total protein and sugar levels were measured by routine methods. The upper limit of normal for protein was 0·45 g/l (NEJM, 1986).

Rabies antibody estimations

Rab was estimated in blood and CSF by the rapid immunofluorescent focus inhibition test (Smith, Yager & Baer, 1973). Tests were made in duplicate, an International Standard was always included and the results expressed in International Units. Rabies-specific IgM (RIgM) was detected by a μ-capture radioimmunoassay technique modified from a method of Japanese encephalitis (JE) IgM assay (Burke, Nisalak & Ussery, 1982). Human diploid cell (HDC) rabies vaccine (Institut Mérieux) was used as rabies antigen in the test and the iodinated conjugate was made with rabbit hyperimmune serum donated by K.G. Nicholson. Sucrose density gradient-separated fractions of serum confirmed the identity of the IgM. The results were expressed as a ratio of the counts of the specimen to negative controls. A ratio of >2 was considered positive. No positive samples contained rheumatoid factor by latex agglutination test. The specificity of the RIgM test was confirmed, and an alternative diagnosis was excluded, by testing all the sera and CSF samples for JE IgM.

Albumin, IgG and IgM measurements

Serum and CSF albumin, IgG and IgM were assayed by radial immunodiffusion in commercially prepared gels (Partigen, Behringwerke) using standard and control sera. More sensitive methods were impractical for potentially rabies-infected specimens. Values of IgM above 3·52 g/l in the serum (NEJM, 1986) and 0·028 g/l in the CSF were considered abnormally raised.

The IgG Index was calculated from the levels of IgG and albumin in the serum and CSF thus:

$$\text{IgG Index} = \frac{\text{IgG}\ \frac{\text{CSF}}{\text{serum}}}{\text{alb}\ \frac{\text{CSF}}{\text{serum}}}$$

a result of $>0{\cdot}85$ indicates intrathecal synthesis of IgG (Thompson, Riches & Kohn, 1983).

The albumin quotient (Q_{alb}) was calculated from serum and CSF albumin levels (Winfield *et al.*, 1983) thus:

$$Q_{alb} = CSF_{alb} \times \frac{10^3}{serum_{alb}}$$

a level of >9 indicates leakage of serum protein across the blood–brain barrier into the CSF.

Rabies diagnostic techniques

Rapid methods of diagnosing rabies encephalitis during life were compared in 17 patients from all three clinical groups with suggestive symptoms and signs. Skin biopsies for immunofluorescent identification of rabies antigen (Bryceson *et al.*, 1975), were taken with a 4 or 6 mm disposable sterile punch, from the neck, including hair follicles and, in five cases, from the bitten limb. Samples were transported on wet ice, embedded in OTC mounting medium (Tissue-Tek OTC compound) on a chuck, and immersed in liquid nitrogen. Seven nm frozen sections were stained with fluorescein-labelled antirabies globulin (BBL) adsorbed with either 20% rabid mouse brain (RMB) or normal mouse brain (NMB) suspensions. A negative control section, stained with RMB-adsorbed conjugate was included on each slide. Sections were scanned under UV light at a magnification of $\times 126$ with a water immersion objective. At least 20, usually more than 50, sections stained with NMB-adsorbed conjugate were examined from each specimen. Control slides were always included.

In 11 cases corneal smears were obtained and stained using the same reagents. Post-mortem brain biopsies from all rabies and one PVE patient were also tested for rabies antigen by the IF test and in seven cases by intracerebral inoculation of suckling mice for viral isolation. Mouse neuroblastoma cells kindly supplied by Dr G.M. Baer were used for virus isolation in one case.

RESULTS

Clinical data

The clinical details of the PVE, rabies and control patients are shown in Table 1. The mean time between starting Semple vaccination and the appearance of symptoms in PVE patients was 15 (range 4–50) days. The mean incubation period of rabies was 12 (range 3–52) weeks. Two of these patients had had HDC or suckling mouse brain (SMB) rabies vaccine. Paraesthesiae or itching at the bite site was often useful in suggesting an early clinical diagnosis of rabies. Fasciculation, usually over the trunk, was seen in both PVE and rabies cases. After 16 days of illness, one PVE patient died in coma with respiratory paralysis. No rabies virus was isolated from the brain and repeated immunofluorescent tests of the brain were negative. Details have been reported elsewhere (Swaddiwuthipong *et al.*, 1988).

CSF and serum samples were taken on two occasions in four PVE cases and on three occasions in one patient. Initial samples were taken between 4 and 135 (median 12) days after the onset of symptoms. All were taken within 4 weeks of the onset except one case in an 8-year-old boy with drowsiness, cranial nerve defects (VII, VI, V) and ataxia. Samples for testing were taken at $4\frac{1}{2}$ and 5 months after onset, and residual neurological impairment lasted at least 7 months.

Table 1. Clinical details of patients

	Rabies	Post-vaccinal encephalitis	Control
No. Patients	10	12	5
No. deaths	10	1	0
Age (years)	37·5 (14–63)*	29 (8–70)	20 (7–35)
Male: Female	8:2	11:1	3:2
Rabies vaccinated	2	12	3

Incidence of clinical features				Diagnoses
Aerophobia/ hydrophobia	10 (100%)	Headache	10	Poliomyelitis
Paraesthesiae/ itching at the bite site	6 (60%)	Impaired consciousness	9	Japanese encephalitis
Fasciculation	3 (30%)	Fever	8	Serum sickness†
		Localized weakness	8	Anxiety†
		Paraesthesiae	4	Reaction to metoclopramide†
		Cranial nerve defects	3	
		Hyporeflexia	3	
		Urinary retention	2	
		Fasciculation	1	

* Mean (range).
† Following rabies vaccination.

Table 2. Results of routine CSF tests

	Rabies	PVE
% Patients with leucocytes	50	58
Mean leucocyte count mm^{-3}	50	57
maximum count	411	351
% Patients with total protein >45 mg/100 ml	30	58
Mean total protein mg/100 ml	22·5	52·8
maximum level	450	136
Mean sugar mg/100 ml	101	73*
range	54–151	23–132

* Only one patient with low level, CSF/blood ratio = 0·37.

Initial samples from rabies patients were taken between 2 and 7 (median 3) days after the onset of symptoms. A second sample was obtained from one patient who survived until the twelfth day.

CSF cell count and total protein
For results see Table 2.

Rabies neutralizing antibody
Rab was detected in the serum of all PVE patients (Table 3). The median level in the serum was 2 iu, and in the CSF, 0·2 iu in eight patients. In contrast, of the 10 rabies patients, only the two who had been vaccinated had detectable serum Rab, and in one antibody was also in the CSF. The serum:CSF antibody ratio varied in PVE patients between 2·8 and 33. The ratio was < 10 in four of the eight cases, and for the rabies patient it was 6·3.

One control patient, who had received Semple vaccine, had 0·95 iu of Rab in his serum. All the other control samples gave negative results.

Rabies-specific IgM
The RIgM test was positive in eight (67%) of PVE patients sera, with a median specimen:control count ratio of 4·6 (range 2·3–7·4). These were the same eight who had had Rab in their CSF. RIgM was also detected in the CSF of seven of these eight cases. The median ratio of counts was 5·3 (range 2·9–11·1). There was no statistical correlation between the level of RIgM and the time after the onset of symptoms, but the higher levels (ratio > 6) were found in specimens taken within 8 days of onset. One sample was still positive after 47 days of illness.

Only one rabies patient, who had had SMB vaccine, had RIgM in both his serum (ratio 7·7) and CSF (ratio 5·1) but the single serum was positive in the unvaccinated patient with Rab on day 15. The RIgM test was negative in all control patients.

JE-specific Igm
JE IgM was found in the serum and CSF of one unconscious control patient with clinical features typical of JE. No JE IgM was detected in any specimen from PVE or rabies patients.

Total IgM
Four PVE patients had raised total serum IgM levels, between 3·7 and 5·0 g/l, and one had a high CSF level of 0·33 g/l. Two of the four patients had no detectable RIgM. The total IgM levels were normal in all rabies patients but serum levels of 4·75 and 3·54 g/l were found in control patients with polio and JE.

IgG Index
Two of the five PVE patients with a high IgG Index had no detectable Rab in their CSF (Table 3), indicating that other antibodies were being produced. The single rabies patient with

Table 3. Serological results on paired serum and CSF samples

	No. of patients	Rabies neutralizing antibody detected (No. of patients)		Rabies specific IgM detected (No. of patients)		Raised* albumin quotient (no. of patients)	Raised† IgG Index (no. of patients)
		Serum	CSF	Serum	CSF		
PVE	12	12 (0·4–9·8)‡	8 (0·1–1·35)‡	8 (2·3–7·4)§	7 (2·9–11·1)	6 (31·9)¶	5 (1·41)¶
Rabies	10	2	1	1	1	3 (59·3)¶	1
Other diagnoses	5	1	0	0	0	0	1

* Indicating blood–brain barrier leakage.
† Indicating intrathecal synthesis of IgG.
‡ Range in International Units.
§ Range of ratio of specimen:control counts.
¶ Maximum value.

Table 4. Rabies diagnosis by antigen detection using immunofluorescence

Diagnosis	No. patients	Skin FAT		Brain FAT		Corneal smear	
		Positive	Negative	Positive	Negative	Positive	Negative
Rabies	11	9	2*	10	1†	0	9
PVE	3	0	3	—	1	0	1
Control‡	3	0	3	—	—	0	1

* One inadequate specimen, the other brain FAT also negative.
† Rabies virus isolated.
‡ Diagnoses: Japanese encephalitis, poliomyelitis, anxiety.

an abnormal IgG index of 0·897 on his fourth day of illness, was only just outside the normal range. There was no Rab detected in his serum or CSF and his Q_{alb} was normal. An increase in IgG Index did not occur at any particular time in the clinical course, raised levels were found at any time after the fourth day of illness. The control JE patient had a high IgG Index of 1·93 as expected.

Albumin quotient

All six PVE patients with a high Qalb also had RIgM in their CSF (Table 3). Two patients had abnormal values for both Qalb and IgG Index. Three rabies patients had elevated Q_{alb} levels (range 9·7–59·3); the two who received vaccine and a man who survived 12 days with intensive care and intrathecal ribavirin therapy had a very high result of 59·3 in the terminal sample, but no evidence of meningitis. There was no correlation between abnormal Q_{alb} and the timing of the samples, nor was there any relation to particular clinical signs or to the severity of illness. As expected, all PVE patients with a high Q_{alb} had raised CSF total protein levels, as did two of the three rabies patients with a high Q_{alb}.

Rapid methods of diagnosing rabies encephalitis

Skin biopsy specimens were taken between 2 and 11 (mean 5) days after the onset of symptoms. Among the 11 patients with rabies positive brain samples, the antigen was detected in the skin biopsy in nine (Table 4). This biopsy method failed to diagnose two cases: in one, the sample was inadequate with no hair follicles, and the neck biopsy from the second patient failed to show rabies antigen despite thorough examination of 35 sections stained with NMB adsorbed conjugate. Repeated brain smears were also negative, but rabies virus was nevertheless isolated in suckling mice. Only 3/16 mice became ill, 14 days after inoculation indicating little virus in the inoculum. The isolate stained well with the same conjugate, so a non-staining variant is unlikely to have caused a false negative result in the fresh smears.

Rabies antigen was not detected in the skin biopsy of the bitten arm of a PVE patient, but the bitten legs of the four rabies cases were all positive. One patient with paralytic (dumb) rabies (not included in the serological study), was bitten on the left foot, with gross weakness of his legs but no hydrophobia. His leg biopsy was positive but no antigen was seen in the neck skin sample. In addition to the IF staining around hair follicles, linear patterns of antigen were seen in the basal layers of the epidermis, in some specimens. In summary: with the single exception of an inadequate specimen, the skin test results were identical to those of the post-mortem brain IF test.

Rabies virus isolation was confirmed by IF, 3 days after inoculation of a brain sample onto mouse neuroblastoma cells.

No rabies antigen was demonstrated in corneal smears from eight rabies patients, one PVE or one poliomyelitis case.

DISCUSSION

Antibody production in the CSF of PVE patients was different to that seen in the animal model of Bell & Moore (1979). Rabies neutralizing antibody was found in the CSF of 67% of PVE patients, and one of two rabies patients who had been vaccinated. The ratio of serum:CSF Rab of 6·3 in the rabies patient was not lower than the PVE group, as might be expected from the mouse data of Bell *et al.* (1966). They found brain neutralizing antibody titres a small fraction ($\simeq 1:10$) of the serum level after vaccination, but comparable ($\simeq 1:2$) levels indicated rabies infection, from which the animals recovered. The Rab levels in our patients were lower than those induced in the three vaccinated human survivors of rabies (Hattwick *et al.*, 1972, Porras *et al.*, 1976, Tillotson, 1977) but in the early stages of disease before the rapid rise in titre, the test would not confirm a diagnosis.

RIgM was found in the CSF of 58% of PVE patients and in one of two vaccinated rabies patients, and therefore it would not be a useful method of differentiating the diseases. No RIgM was detected in the CSF of unvaccinated humans with rabies in this study. It was, however, found at the onset of symptoms in the CSF of rabid dogs, assayed in the same laboratory (Tingpalapong *et al.*, 1986).

Leakage of rabies neutralizing antibody across the blood–brain barrier has been reported in dogs with non-fatal rabies encephalitis, but not in hyperimmune animals (Gough *et al.*, 1974). The non-specific pathological changes of encephalitis may permit this leakage which we also found in six of seven PVE patients with RIgM in the CSF.

Semple vaccine sometimes induces serum antibodies reacting with human brain extract (Kirk & Ecker, 1949), and the highest levels are found in patients with neuroparalytic reactions (Koprowski & Le Bell, 1950). Our results indicated intrathecal IgG synthesis in 5 of 12 PVE patients, which was neither quantitatively nor qualitatively associated with Rab in the CSF. This suggests that other IgG antibodies were being produced in the CNS, and is in keeping with a recent study in a similar group of Thai PVE patients showing evidence of intrathecal antibody production to myelin basic protein in 36% of cases (Hemachudha *et al.*, 1987).

One PVE patient developed symptoms 50 days after her first dose of vaccine, but only 17 days following the last booster dose. Although an alternative diagnosis of her drowsiness and predominantly left-sided weakness cannot be excluded with certainty, such a late onset of PVE is not unique. In a Japanese series, symptoms commonly began between 5 and 8 weeks after the first inoculation (Shiraki *et al.*, 1962).

The absence of a detectable rabies neutralizing antibody response in the first week of illness in unvaccinated rabies patients is consistent with other reports (Hattwick & Gregg, 1975).

Intrathecal synthesis of non-specific IgM was reported during the second and third week of illness in three unvaccinated rabies patients (Schuller *et al.*, 1979). We found normal serum and CSF levels of total IgM in eight unvaccinated patients but, with one exception, the samples were taken in the first week.

The two vaccinated rabies patients had leakage across the blood–brain barrier in the first week of illness unlike the nine unvaccinated ones. This may be a clue to a different pathogenesis of the encephalitis following vaccination, since the replication of the virus *in vitro* is enhanced by the presence of small amounts of antibody (King, Sands & Porterfield, 1984).

A negative rabies IF test on animal brains, when the virus is isolated in suckling mice, occurs in about 2% of specimens, for a variety of reasons (Kissling, 1975). In humans, the part of the brain sampled is unlikely to include the optimal areas. The pattern of IF staining in the epidermis of the skin biopsies is similar to that demonstrated in mice (Correa-Giron, Allen & Sulkin, 1970). Bryceson *et al.*, (1975) first used this method in man and Blenden, Creech & Torres-Anjel (1986) reported 60–100% success in diagnosing rabies, depending on the stage of the illness. Other isolated case reports have been less successful (Anderson *et al.*, 1984). In our hands, the skin biopsy technique is the best way of diagnosing rabies in life with no false positive results, but including unexpected diagnoses of paralytic rabies (Phuapradit *et al.*, 1985). It gave identical results to the post-mortem brain smear examination, providing that the skin sample included hair follicles.

Neither neutralizing antibody nor RIgM tests are useful in diagnosing rabies or PVE in the crucial first week of illness. In future, techniques to detect antibodies to myelin basic protein may be adapted to routine use for the diagnosis of most cases of PVE (Hemachudha *et al.*, 1987). In the meantime, a diagnosis of PVE is strongly suggested in patients at risk if the skin biopsy shows no rabies antigen.

ACKNOWLEDGMENTS

We thank Professor Khunying Tranakchit Harinasuta and Professor Danai Bunnag for their support; Dr G.S. Turner for advice; the medical and nursing staff of Bamrasnaradura and Ramathibodi Hospitals for their cooperation; Dr Rapin Sanitphan and Mrs Vanaporn Wuthiekanun for expert technical work; Dr Juntra Karbwang for her clinical assistance and Miss Eunice Berry and Mrs Elwena Gregory for typing. This study was part of the Wellcome-Mahidol University, Oxford Tropical Medicine Research Programme, funded by the Wellcome Trust of Great Britain.

REFERENCES

ANDERSON L.J., NICHOLSON, K.G., TAUXE, R.V. & WINKLER, W.G. (1984) Human rabies in the United States 1960 to 1979: Epidemiology, diagnosis and prevention. *Ann. int. Med.* **100,** 728.

ARKO, R.J., SCHNEIDER, L.G. & BAER, G.M. (1973) Non fatal canine rabies. *Am. J. Vet. Res.* **34,** 937.

ATANASIU, P., SAVY, V. & GIBERT, C. (1978) Rapid immunoenzymatic technique for titration of rabies antibodies IgG and IgM results. *Med. microbiol. Immunol.* **166,** 201.

BELL, J.F., LODMELL, D.L., MOORE, G.J. & RAYMOND, G.H. (1966) Brain neutralization of rabies virus to distinguish recovered animals from previously vaccinated animals. *J. Immunol.* **97,** 747.

BELL, J.F. & MOORE, G.J. (1979) Allergic encephalitis, rabies antibodies, and the blood/brain barrier. *J. Lab. clin. Med.* **94,** 5.

BLENDEN, D.C., CREECH, W. & TORRES-ANJEL, M.J. (1986) Use of immunofluorescence examination to detect rabies virus antigen in the skin of humans with clinical encephalitis. *J. infect. Dis.* **154,** 698.

BRYCESON, A.D.M., GREENWOOD, B.M., WARRELL, D.A., DAVIDSON, N.Mc.D., POPE, H.M., LAWRIE, J.H., BARNES, H.J., BAILIE, W.E. & WILCOX, G.E. (1975) Demonstration during life of rabies antigen in humans. *J. infect. Dis.* **131,** 71.

BURKE, D.S., NISALAK, A. & USSERY, M.A. (1982) Antibody capture immunoassay detection of Japanese encephalitis virus immunoglobulin M and G antibodies in cerebrospinal fluid. *J. clin. Microbiol.* **16,** 1034.

BURKE, D.S., NISALAK, A., USSERY, M.A., LAORAKPONGSE, T. & CHANTAVIBUL, S. (1985) Kinetics of IgM and IgG responses to Japanese encephalitis virus in human serum and cerebrospinal fluid. *J. infect. Dis.* **151,** 1093.

CORREA-GIRON, E.P., ALLEN, R. & SULKIN, S.E. (1970) The infectivity and pathogenisis of rabies virus administered orally. *Amer. J. Epidemiol.* **91,** 203.

DEAN, D.J. & ABELSETH, M.K. (1973) The fluorescent antibody test. In *Laboratory Techniques in Rabies. 3rd edition* (ed. by M.M. Kaplan & H. Koprowski), pp. 73–84. WHO, Geneva.

GOUGH, P.M., DIERKS, R.E., RUSSELL, R.M. & ARCHER, B.G. (1974) Characterization of brain-associated rabies neutralizing substance. *J. infect. Dis.* **129,** 456.

HATTWICK, M.A.W. & GREGG, M.B. (1975) The disease in man. In *The Natural History of Rabies Vol. II* (ed. by G.M. Baer), p. 281. Academic Press, New York.

HATTWICK, M.A.W., WEIS, T.T., STECHSCHULTE, C.J., BAER, G.M. & GREGG, M.B. (1972) Recovery from rabies, a case report. *Ann. int. Med.* **76,** 931.

HEMACHUDHA, T., GRIFFIN, D.E., GIFFELS, J.J., JOHNSON, R.T., MOSER, A.B. & PHANUPHAK, P. (1987) Myelin basic protein as an encephalitogen in encephalomyelitis and polyneuritis following rabies vaccination. *New Eng. J. Med.* **316,** 369.

KING, A.A., SANDS, J.J. & PORTERFIELD, J.S. (1984) Antibody-mediated enhancement of rabies virus infection in a mouse macrophage cell line (P388D1). *J. Gen. Virol.* **65,** 1091.

KIRK, R.C. & ECKER, E.E. (1949) Time of appearance of antibodies to brain in the human receiving anti-rabies vaccine. *Proc. Soc. exp. Med.* **70,** 734.

KISSLING, R.E. (1975) The fluorescent antibody test in rabies. In *The Natural History of Rabies Vol. I* (ed. by G.M. Baer), p. 401. Academic Press, New York.

KOPROWSKI, H. & LE BELL, I. (1950) The presence of complement-fixing antibodies against brain tissue in sera of persons who had received antirabies vaccine treatment. *Am. J. Hyg.* **51,** 292.

NEW ENGLAND JOURNAL OF MEDICINE (1986) Normal reference laboratory values. *New Eng. J. Med.* **314,** 39.

PHUAPRADIT, P., MANATSATHIT, S., WARRELL, M.J. & WARRELL, D.A. (1985) Paralytic rabies: some unusual presentations. *J. Med. Assoc. Thai.* **68,** 106.

PORRAS, C., BARBOZ, J.J., FUENZALIDA, E., ADAROS, H.L., OVIEDO DE DIAZ, A.M. & FURST, J. (1976) Recovery from rabies in man. *Ann. int. Med.* **85,** 44.

ROUMIANTZEFF, M., AJJAN, N., MONTAGNON, B. & VINCENT-FALQUET, J.C. (1985) Rabies vaccines produced in cell culture. *Ann. inst. Past. Virol.* **136E,** 413.

SCHULLER, E., HELARY, M., ALLINQUANT, B., GIBERT, C., VACHON, F. & ATANASIU, P. (1979) IgM and IgG antibody responses in rabies encephalitis. *Ann. Microbiol. (Inst. Pasteur)* **130A,** 365.

SHIRAKI, H., ONTANI, S., TAMTHAI, B., CHAMNUNI, A., CHITANONDH, H. & CHARUCHINDA, S. (1962) Rabies postvaccinal encephalomyelitis and genuine rabies in human beings. *World Neurol.* **3,** 125.

SMITH, J.S., YAGER, P.A. & BAER, G.M. (1973) A rapid reproducible test for determining rabies neutralizing antibody. *Bull. WHO,* **48,** 535.

SWADDIWUTHIPONG, W., WENIGER, B.G., WATTANASRI, S. & WARRELL, M.J. (1988) A high rate of neurological complications following Semple anti-rabies vaccine. *Trans Roy. Soc. Trop. Med. Hyg.* In press.

THOMPSON, E.J., RICHES, P.G. & KOHN, J. (1983) Antibody synthesis within the central nervous system: comparison of CSF IgG indices and electrophoresis. *J. clin. Pathol.* **36,** 312.

TILLOTSON, J.R. (1977) Rabies in a laboratory worker—New York. *Morbid. Mortal. wkly Rep.* **26,** 183.

TINGPALAPONG, M., HOKE, C.H., WARD, G.S., BURKE, D.S., ELWELL, M.R., LOHYTYOTHIN, S. & SAISOMBAT, S. (1986) Anti-rabies virus IgM in serum and cerebrospinal fluid from rabid dogs. *Southeast Asian J. Trop. Med. Pub. Hlth,* **17,** 550.

WINFIELD, J.B., SHAW, M., SILVERMAN, L.M., EISENBERG, R.A., WILSON, H.A. & KOFFLER, D. (1983) Intrathecal IgG synthesis and blood–brain barrier impairment in patients with systemic lupus erythematosus and central nervous system dysfunction. *Am. J. Med.* **74,** 837.