Rabies encephalitis

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INTRODUCTION
It is possible to provide very efficient prophylaxis against rabies encephalitis, which remains universally fatal in practice. The unique pathogenesis of this neurotropic virus is slowly being unravelled, but the relevance of the paradoxical immunological features remain mysterious. The epidemiology of rabies is being clarified because virus strains can now be classified and identified precisely. Recent developments are reviewed here; also a clinician's view of rabies, its diagnosis, and of the patients said to have recovered from rabies encephalitis.

Rabies is a zoonosis of certain mammal species, endemic in all continents. Only a few European countries, some islands and peninsulas and Antarctica, are free of the fear of rabies, although imported infection is a universal risk. Rabies occurs in separate cycles within dogs and wild mammal vector species (see 'Post-exposure treatment', page 24), and the virus sometimes spills over to nonvector species such as humans. Strains of virus from different animals and geographical areas can be identified by genetic sequence analysis, or by antigenic typing using a panel of monoclonal antibodies. The urban enzootic in domestic dogs is of most importance to man, and is the cause (World Health Organization 2000). In the USA, where sylvatic (wildlife) rabies is endemic, there have been 32 deaths over 10 years since 1990. Of these, 24 (75%) were due to bat rabies viruses, and only two reported a bite, although half had had recognized contact with a bat (Centers for Disease Control 2000).

The lyssaviruses
The bullet-shaped rabies virion contains a single strand of RNA of negative polarity. A ribonucleoprotein complex core of the virus is covered by a glycoprotein coat bearing projecting spikes (King & Turner 1993). Rabies is the first of seven genotypes, part of the large Rhabdoviridae family (lyssa = Gk, frenzy). The other six genotypes are rabies-related viruses (Badrane et al. 2001), of which have caused fatal infection (Warrell et al. 1995; Hooper et al. 1997). Two genotypes (5 and 6) are European bat lyssaviruses found in insectivorous bats across Europe. Australian bat lyssavirus (genotype 7) was discovered in flying foxes (fruit bats, genus Pteropus), in 1996 (Hooper et al. 1997). Two further genotypes (3 and 4), Mokola virus in shrews and cats, and Duvenhage virus in bats, are very occasionally found but only in Africa (King & Turner 1993; Warrell et al. 1995). The Lyssavirus genus has recently been divided into two phylogroups as a result of serological and genetic analyses (Badrane et al. 2001). Phylogroup I comprises all these viruses except Mokola virus, which is in phylogroup II with Lagos Bat virus, which has not been found in humans. All phylogroup I genotypes have caused fatal rabies-like encephalitis in of more than 95% of human rabies cases.

The incidence of rabies is unknown due to the gross under-reporting of an untreatable disease whose victims often choose to die at home, to avoid the expense of medical care. The highest recognized human mortality is in Asia. In India 30 000 deaths (3/105 population) were reported to WHO in 1998 man, whereas Mokola virus has probably only caused three known human infections, one of which was a fatal encephalitis without any typical features of rabies (Warrell et al. 1995). Experimentally, phylogroup II viruses are less pathogenic, and there is little if any cross-neutralization with the phylogroup I lyssaviruses. This is of practical relevance for post-expo-
sure treatment (see page 24). No rabies-related viruses have yet been found in the Americas.

PATHOGENESIS

Virus transport to the central nervous system (King & Turner 1993; Charlton et al. 1994)

The recent progress in molecular neurophysiology has begun to reveal the intriguing journey of the neurotropic rabies virus into and through the nervous system, and its eventual distribution to many organs. The virus is inoculated into tissues of a wound and it may replicate locally in muscle cells or attach directly to nerve endings, in particular to nicotinic acetylcholine receptors at motor endplates. This competitive binding is blocked by a snake venom neurotoxin, -bungarotoxin, whose structure has a sequence homologous with the rabies glycoprotein molecule. Attachment also occurs to several other neuronal receptors (Thoulouze et al. 1998). The virus then enters the presynaptic nerve ending by endocytosis and may be associated with synaptic vesicles (Lewis et al. 2000). Inside peripheral nerves, the virus is carried in a retrograde direction by fast axonal transport (Kelly & Strick 2000) centripetally to the central nervous system (CNS). This progression can be blocked experimentally by local anaesthetics, metabolic inhibitors and nerve section. Two independent laboratories (Jacob et al. 2000; Raux et al. 2000) have recently found that the rabies phosphoprotein interacts with LC8 protein. This molecule is highly conserved across species. One of the roles of LC8 is as a component of the cargo-binding complex on the microtubule dynein motor, which operates the retrograde organelle axonal transport system. This association is evidence in support of the deduced viral pathway. At this stage, rabies antigen is undetectable in vivo, perhaps due to the small number of virions, or naked nucleocapsids, involved. The virus remains intraneural throughout its passage and is experimentally inaccessible to extraneural antibodies.

Dissemination of virus in the central nervous system

On reaching the CNS, there is massive viral replication on membranes within neurones and transsynaptic transmission of virus occurs from cell to cell. Glial cells are very rarely infected. Viral proteins accumulate in the cytoplasm appearing as inclusions, the classical Negri Bodies (Fig. 1). Virions are now visible by electron microscopy in neurones (Charlton et al. 1994). Involvement of the limbic system and amygdaloid nuclei cause aggressive behaviour in animals. Hydrophobia in man is probably associated with brain stem infection (Warrell et al. 1976). Despite minimal if any pathological changes in some patients, there is gross neuronal dysfunction. The mechanism is unknown but there are a variety of EEG changes, and experimental evidence of defects at various stages of neurotransmitter activity in the serotonin, opiate, GABA and muscarinic acetylcholine synapses (Charlton et al. 1994; Tsiang 1993). Rabies infection may also affect transmembrane ion channel activ-

Figure 1 Negri body (arrow) in a Purkinje cell of the cerebellum. (Courtesy of F A Murphy.)
ity. Changes in the functional expression of some channels, and attenuation of inhibition of others, have been shown in vitro (Iwata et al. 2000). As there is usually no detectable immune response at the onset of illness, significant immunopathology is unlikely.

**Centrifugal spread of virus from the central nervous system**

Retrograde axonal transport from the brain, via somatic and autonomic efferent nerves, deposits virus in many tissues (Helmick et al. 1987; Dueñas et al. 1973; Jackson et al. 1999) including: skeletal and cardiac muscle, adrenal medulla where infection may be clinically significant, kidney, taste buds, respiratory tract, cornea, and nerve twiglets in the hair follicles (see ‘Diagnosis of human rabies encephalitis during life’). At this stage, productive viral replication occurs, with budding from outer cell membranes in the salivary, lacrimal and other glands, which permits the further transmission of rabies by bites to other mammals. There is no evidence of viraemia, but rabies virus is shed in human saliva, lacrimal and respiratory tract secretions, rarely in urine (Anderson et al. 1984) and possibly in milk.

**Immunology**

Rabies virus evades recognition by the immune system until this late stage of the disease. At the site of inoculation, some virus may be briefly exposed, but once within neurons, virions and their antigens are hidden from immune surveillance. When virus is eventually secreted, rabies antigens are expressed on cell surfaces but the virus is then widely distributed throughout the body and an immune response is too late to combat the overwhelming infection. Neutralizing antibody usually appears during the second week of illness in unvaccinated patients, and remains at low levels until death a few days later (Anderson et al. 1984). Evidence of cell-mediated immunity, by lymphocyte transformation tests, was found in six of nine furious encephalitis patients, but not in seven with paralytic disease (Hemachudha et al. 1988), who had few B cells. Both groups had reduced NK cell levels (Sriwanthana et al. 1989).

Studies of encephalitis in rodents show that survival or delayed mortality are associated with the early appearance of IFN-α in the brain and neutralizing antibody (Consales et al. 1990; Camelo et al. 2000; Hooper et al. 1998), and also inflammation and up-regulation of MHC class II mRNA expression in CNS cells (Irwin et al. 1999). Infected human brains show remarkably little pathology, so apoptosis is unlikely to be a significant cause of death, which is in keeping with experimental findings.

The ‘early death’ phenomenon

It has long been recognized that the rabies incubation period is shorter than average in patients who were inadequately vaccinated and develop rabies. This effect can be produced experimentally by injecting a little rabies immune serum or B cells during the incubation period. This immune enhancement or ‘early death’ phenomenon seems similar to that observed with dengue and other viruses (Prabhakar & Nathanson 1981).

**Rabies nucleoprotein superantigen**

Another recognized phenomenon is the nonspecific immunosuppressive effect of rabies infection. One possible explanation is the finding that rabies nucleoprotein acts as a weak superantigen because it directly induces proliferation of human CD4 Th2 cells bearing the V8 TCR (Lafon 1997). The effect of this superantigen in human disease is unknown, but it possibly immunosuppresses by stimulating an inefficient polyclonal antibody response and so prevents an effective specific immune response.

**Antiviral treatments**

Antivirals have not been successful against rabies but a noncompetitive antagonist of
NMDA, ketamine, was found to inhibit rabies viral replication in vitro and in vivo. This surprising effect works through temporary specific inhibition of rabies virus genome transcription without suppressing host cell transcription (Lockhart et al. 1992). The only experimental treatment capable of clearing a lethal dose of virus from infected mouse brain is one particular monoclonal antibody, which does not have high neutralizing activity but appears to restrict virus spread from cell to cell and to restrict virus gene expression (Dietzschold et al. 1992).

CLINICAL FEATURES
Infection usually results from inoculation of virus in an animal’s saliva through the skin or onto mucous membranes and rarely by inhalation of aerosolized virus or via infected corneal transplants. The interval between inoculation and the onset of symptoms is usually between 20 and 90 days, but it has varied from 4 days to 19 years (Smith et al. 1991). In general, the nearer the bite to the head, the shorter the incubation period.

Prodromal symptoms
Itching or paraesthesiae at the site of the healed bite wound are the only specific prodromal symptoms, occurring in about 40% of patients. Non-specific features include: fever, headache, myalgia, fatigue, sore throat, gastrointestinal symptoms, irritability, anxiety and insomnia. Psychiatric disease may be suspected. The disease progresses to either furious or paralytic rabies encephalitis, usually within a week (Warrell 1976).

Furious rabies
This well-known form is the most easily recognized. Malfunction of the brain stem, limbic system and higher centres results in the characteristic hydrophobic spasms. This is a reflex contraction of inspiratory muscles provoked by attempts to drink water, and later by even the sound or mention of water, and

Figure 2 Progression of a hydrophobic spasm associated with terror in a Nigerian boy with furious rabies (a,b). Note the initial powerful contraction of the diaphragm (depressing the xiphisternum) and sternocleidomastoid muscles. (c) The episode terminates in opisthotonos. (© Copyright D. A. Warrell.)
also sometimes by draughts of air (aerophobia), touching the palate, bright lights or loud noises (Fig. 2).

Intense thirst forces patients to try to drink. They may have a tight feeling in the throat, the arm tremble, and jerky spasms of the sternomastoid, diaphragm and other inspiratory muscles lead to a generalized extension, sometimes with convulsions and opisthotonos (Warrell 1976). There is an associated inexplicable feeling of terror, which occurs during the first episode, and is not a learned response, but may be reinforced by conditioning (Warrell et al. 1976). Respiratory or cardiac arrest following a hydrophobic spasm is fatal in one-third of cases.

Excitation, aggression, anxiety or hallucinations occur between calm, lucid intervals, when no neurological abnormality may be detectable. Other features include cardiac arrhythmias, myocarditis, labile blood pressure and temperature, respiratory disturbances (e.g. Cheyne-Stokes respiration, cluster breathing), meningism, III, VII and IX cranial nerve palsies, abnormal pupillary responses, muscle fasciculation (Warrell 1976), autonomic stimulation with lacrimation and salivation and rarely increased libido, priapism and spontaneous orgasms, suggesting involvement of the amygdaloid nuclei as in Kluver-Bucy syndrome. Coma eventually ensues, with flaccid paralysis, and the illness rarely lasts more than a week without intensive care.

**Paralytic rabies**

Less common than furious rabies, paralytic or ‘dumb’ rabies may be missed unless there is a high level of suspicion. Paralytic disease is characteristic of vampire bat-transmitted rabies and is more common following infections by attenuated viruses, and perhaps after post-exposure vaccination (Warrell et al. 1995).

Prodromal symptoms are followed by paraesthesiae or hypotonic weakness, commonly starting near the site of the bite and spreading cranially. Fasciculation, myoedema or piloerection may be seen. The ascending paralysis results in constipation, urinary retention, respiratory failure and inability to swallow. Flaccid paralysis, especially of proximal muscles, is associated with loss of tendon and plantar reflexes, but sensation is often normal. Hydrophobic spasms may occur in the terminal phase and death ensues after 1–3 weeks (Warrell 1976).

**Differential diagnosis**

Rabies should be suspected if inexplicable neurological, psychiatric or laryngopharyngeal symptoms occur in those who have been to an endemic area. The animal contact may have been forgotten, never observed in small children or subtle and unnoticed as in the case of bat bites. The differential diagnoses include (Warrell 1976) the cephalic form of tetanus, which usually has an incubation period less than 15 days, constant muscle rigidity, and normal CSF. Other conditions are intoxications including delirium tremens, poisoning, Guillain-Barré syndrome presenting as paralytic rabies, or very rarely following rabies tissue culture vaccine treatment. Rabies phobia is a hysterical response, usually very soon after a bite, with aggressive behaviour and an excellent outcome. Other viral encephalitides, including Japanese encephalitis, poliomyelitis and treatable Herpes simiae B from a monkey bite, should be considered. In recipients of nervous tissue-containing rabies vaccines, postvaccinal encephalitis can be clinically indistinguishable from paralytic rabies.

**Management**

Patients with rabies must be sedated heavily and given adequate analgesia to relieve their terror and pain. In the absence of specific treatment, once the diagnosis has been confirmed there is little justification for intensive care because no one is known to have survived furious rabies encephalitis. Patients given intensive care develop complications such as cardiac arrhythmias, cardiac and respiratory failure, raised intracranial pressure, convulsions, fluid and electrolyte disturbances including diabetes insipidus and inappropriate secretion of antidiuretic hormone, and hyperpyrexia. Antiviral agents including intrathecal ribavirin (ribavirin) and interferon-α, rabies immunoglobulin, corticosteroids and other immunosuppressive drugs have proved useless in man (Warrell et al. 1995).
Survival after rabies encephalitis and differential diagnosis from neurological reactions to rabies vaccines

Animals of several species including the natural rabies vectors, bats, skunks, mongooses and very rarely dogs have recovered from rabies (King & Turner 1993). In Brazil, 11% of wild mammals were recently found to be seropositive (Almeida et al. 2001). Although humans with paralytic rabies can survive for several weeks, especially with intensive care (Emmons et al. 1973) the illness progresses relentlessly. However, four patients, over the last 30 years, have been claimed as survivors of encephalitis. None had classical hydrophobia, and no virus or viral antigen was detected in any patient. The diagnoses were based on finding high rabies neutralizing antibody levels in the CSF.

Two of the patients were treated post-exposure with rabies vaccines of nervous tissue origin. An Argentine woman (Porras et al. 1976) was bitten by her clinically rabid dog, and began a course of suckling mouse brain vaccine 10 days later. Three weeks after the bite she had paraesthesia of the bitten arm, with tremors, myoclonic spasms, ataxia and other signs of cerebellar dysfunction. She recovered within a month but relapsed on two occasions with increasing severity following booster doses of rabies vaccine. Hypertonic tetraparesis, dysphonia and dysphagia developed with varying levels of consciousness. An ECG showed a cardiac conduction defect. Spasticity and tremor resolved slowly over a year. The CSF neutralizing antibody level three months after onset was 1 : 160 000, when the serum level was only 1 : 41 800. Typical clinical features of rabies include the subjective paraesthesiae of the bitten limb, the cardiac conduction defect (if it was a new finding) and the high antibody level. The deterioration following repeated doses of the vaccine suggests postvaccinal encephalitis (Vejajiva 1968). Myoclonus, tremor and cerebellar signs are atypical of paralytic rabies (Hemachudha & Phuapradit 1997), whereas ataxia and tremor is seen with PVE (Applebaum & Greenberg 1953; Rubin et al. 1973). The rabies antibody response to nervous tissue vaccines is higher than usual in patients with severe PVE (Hemachudha et al. 1989).

The second patient was a nine year old boy in USA (Hattwick et al. 1972) who was bitten by a proven rabid bat on the thumb, and was treated with duck embryo vaccine beginning the following day. After 20 days he developed a meningitic illness progressing to encephalitis with unilateral weakness maximal in the bitten arm. Focal seizures and coma ensued, lasting more than a week. The ECG showed atrial arrhythmias. Prolonged intensive care resulted in complete recovery in six months. The CSF antibody level was 1 : 3200 and in the serum 1 : 6300 two weeks after onset. The serum antibody level rose to 1 : 32 700 at two months. Features suggesting rabies are the dominant signs in the bitten limb, cardiac arrhythmias and the high antibody level. However, weakness of one hand progressing to hemiplegia occurred during a reaction to Semple vaccine (Hemachudha et al. 1987). The various neurological complications of duck embryo vaccine have been reviewed (Label & Batts 1982).

The third case was a laboratory worker in New York (Centers for Disease Control 1977a; Centers for Disease Control 1977b) who was thought to have inhaled an aerosol of a fixed strain of rabies (SAD) virus. He had had regular pre-exposure duck embryo rabies vaccine, but none recently. Fever and nonspecific encephalitic symptoms, spastic hemiparesis, myoclonus, impaired consciousness and respiratory arrest developed over two weeks. Hypertension followed, and gradual improvement began at two months, but a personality disorder and dementia became apparent. Two and a half years later he still had profound neurological deficits. Six weeks after onset the CSF neutralizing antibody level was 1 : 22 700 and the parallel serum level 1 : 152 000.

Four patients over the last 30 years have been claimed as survivors of encephalitis. None had classical hydrophobia, and no virus or viral antigen was detected in any patient.
The fourth case, a Mexican boy severely bitten on the head by a proven rabid dog (Alvarez et al. 1994), was given a course of vero cell vaccine, starting the following day, but no rabies immune globulin (RIG) treatment. Nineteen days later he developed fever, signs of encephalitis and convulsions. Intracranial hypertension and coma ensued. After three weeks he improved, reacted to painful stimuli and no longer needed respiratory support. But quadriplegia persisted, he became blind and deaf and eventually died after two years and 10 months. After three weeks of illness, the CSF rabies antibody titre was 1 : 78 125 and 1 : 34 800 in the serum. A second boy with similar clinical features survived at least nine months (Alvarez et al. 1996).

Case reports of neurological illness following human diploid cell rabies vaccine describe either a Guillain–Barré-like syndrome (Boe & Nyland 1980; Bernard et al. 1982; Knittel et al. 1989), a developing mild hemiplegia (Tornatore & Richert 1990), or three patients with symptoms restricted to an arm, possibly the injection site (Gardner & Pattison 1983) (one followed a fetal bovine kidney cell vaccine, now no longer produced) (Courrier et al. 1986). The incidence of neurological disease after these rabies vaccines is no more than after other commonly used vaccines.

Rabies virus was not isolated nor was antigen identified in any of these patients. False negative results may have occurred because the samples were taken late, when there was already a high titre of antibody neutralizing the virus or covering the epitopes of the antigens. The diagnosis of rabies in similar cases might be confirmed in future, now there are sensitive PCR techniques for antigen detection.

The diagnosis of postvaccinial encephalitis (PVE) is made by exclusion of rabies or other diseases. It is an autoimmune response stimulated by the injected neural antigens principally myelin basic protein. B-cell epitopes of this protein, which induce immunity in PVE patients, have been identified and have revealed possible mechanisms of pathogenic cross-reactive immunity against myelin oligodendrocyte protein, which induces demyelination experimentally (Piyasirisilp et al. 1999). A positive diagnosis of PVE may be possible in the future.

Unfortunately this problem of PVE is not just of historical interest. Although modern tissue culture vaccines have been used exclusively in Western Europe and North America for more than 20 years, nervous tissue vaccines are still used in many countries where dog rabies is endemic. Recent annual estimates of the use of Semple vaccine, a homogenate of sheep brain, are: 50 000 people a year in Bangladesh and also in Pakistan; 450 000 in India; 25 000 in Nepal. Suckling mouse brain vaccine is used in Vietnam (500 000 people a year), in Indonesia, North Africa, Mexico, Brazil and other countries of South America. The incidence of neurological symptoms following treatment varies with different products, but is up to 1 : 220 recipients of Semple vaccine, with a mortality rate of 3% (Swaddiwuthipong et al. 1987). Symptoms usually appear within two weeks of starting the course, but may not appear until two months later. Suckling mousebrain vaccines have a lower complication rate [1 : 8000 (Held & Lopez Adaros 1972) to 1 : 27000 (Larghi et al. 1981)] but peripheral nervous system signs usually predominate and the mortality is 22% (Held & Lopez Adaros 1972).

The wide variety of neurological signs include polyneuritis often involving limbs, transverse myelitis, ascending paralysis and meningoencephalitis (Applebaum & Greenberg 1953; Swamy et al. 1984). Corticosteroid therapy (prednisolone 40–60 mg/day) is conventional, and cyclophosphamide in addition has been suggested (Swamy et al. 1984). Recovery often occurs within two weeks and is usually complete, but neurological deficits can ensue.

**DIAGNOSIS (TABLE 1)**

**Diagnosis of human rabies encephalitis during life**

In most parts of the world, a diagnosis of rabies is made clinically, and viral identification is not attempted. In developed countries it should be possible to confirm rabies by virus isolation, rapid identification of antigen or, in unvaccinated people, antibody detection. It is important to take serial samples for the recommended tests as soon as the diagnosis is considered, because confirmation will guide
the appropriate management of the patient, relatives and staff, and allow characterization of the virus.

**Isolation of rabies virus**

Culture of the virus from saliva, throat, tracheal or eye swabs, brain biopsy samples, and possibly CSF is most successful during the first week of illness (Anderson et al. 1984). Isolation of virus in tissue culture of murine neuroblastoma cells can take only one or two days (Bourhy & Sureau 1990), but the method of inoculation of suckling mice yields results in one to three weeks.

**Antigen detection** (Bourhy & Sureau 1990; Bourhy et al. 1998)

The most rapid means of diagnosing rabies during life is by immunofluorescent antibody (IFA) identification of antigen in a skin biopsy. A full thickness biopsy, preferably taken with a disposable biopsy punch, must include the bases of hair follicles. It is taken from a hairy area, usually the nape of the neck, and in addition near the original bite wound if there is an adjacent hairy area. Vertical frozen sections through skin indicate rabies antigen in the nerve twiglets around the base of hair follicles, in a characteristic pattern (Bryceson et al. 1975). Careful controls of specificity are needed, but this method is 60–100% sensitive (Warrell et al. 1988; Blenden et al. 1986). False positives have not been reported.

Antigen detection by IFA in the corneal smear test is too insensitive to be useful (Anderson et al. 1984; Warrell et al. 1988; Crepin et al. 1998) and false positives have occurred.

PCR techniques now seem to be a reliable means of identifying and genotyping rabies strains in a few reference laboratories (Noah et al. 1998). The saliva, CSF (Crepin et al. 1998) and even skin biopsy have proved suitable samples for antigen detection.

**Antibody detection**

In unvaccinated patients, rabies seroconversion often occurs during the second week of illness and is diagnostic. Antibody may be detectable in the CSF a few days later. A mild pleocytosis is only seen in 60% of patients in the first week (Anderson et al. 1984). In vaccinated people, very high levels of antibody in the serum, and especially in the CSF, have been used to make the diagnosis (Hattwick et al. 1972).

**Postmortem diagnosis in humans**

Virus isolation from secretions is usually unsuccessful after two weeks of illness, but culture of brain tissue should still be possible postmortem, even if the IFA staining is negative. Samples can be obtained without a full postmortem examination. Brain necropsies are taken with a Vim–Silverman or other long biopsy needle via the medial canthus of the eye, through the superior orbital fissure; via the nose through the ethmoid bone; by an occipital approach through the foramen magnum; or, through burr holes or an open fontanelle in children.

A retrospective diagnosis using formalin-fixed brain specimens is possible by trypsin digestion and labelled antibody staining with immunofluorescent (Swoveland & Johnson 1983) or enzymatic (Fekadu et al. 1988) techniques.

**Diagnosis in the biting mammal**

If laboratory facilities are available, suspect rabid animals should be killed immediately.

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**Table 1** Human Rabies Diagnosis

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>AIM</th>
<th>TEST</th>
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<tbody>
<tr>
<td><strong>DURING LIFE</strong></td>
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<tr>
<td>SKIN PUNCH BIOPSY</td>
<td>ANTIGEN DETECTION</td>
<td>IMMUNOFLOUORESCENCE TEST</td>
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<tr>
<td>SALIVA, TEARS, CSF</td>
<td>VIRUS ISOLATION</td>
<td>TISSUE CULTURE</td>
</tr>
<tr>
<td>Serum, CSF</td>
<td>ANTIGEN DETECTION</td>
<td>MOUSE INOCULATION TEST</td>
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<tr>
<td></td>
<td>SEROLOGY</td>
<td>PCR</td>
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<td></td>
<td></td>
<td>NEUTRALIZING ANTIBODY</td>
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<td></td>
<td>UNVACCINATED TEST IMMEDIATE</td>
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<td></td>
<td></td>
<td>VACCINATED, SAVE FOR COMPARISON LATER</td>
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</tbody>
</table>

Repeat samples frequently until diagnosis made

**POST-MORTEM**

If no diagnosis yet confirmed – take all above samples

If diagnosis only by antigen detection – isolate virus

<table>
<thead>
<tr>
<th>BRAIN: NEEDLE BIOPSY, OR FULL POST MORTEM SAMPLES</th>
<th>ANTIGEN DETECTION</th>
<th>IF TEST</th>
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<tbody>
<tr>
<td></td>
<td>PCR</td>
<td>TISSUE CULTURE</td>
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<td></td>
<td></td>
<td>MOUSE INOCULATION TEST</td>
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and their brains tested for rabies infection (World Health Organization 1997). Observation in captivity is potentially dangerous and uncertain.

RABIES PROPHYLAXIS

Rabies vaccines
Two rabies vaccines are now licensed for use in the UK – Human diploid cell vaccine (HDCV) (Aventis Pasteur, Lyon, France) and purified chick embryo cell (PCEC) vaccine (RabipurTM; Chiron Behring, Marburg, Germany). Both are in 1 mL dose vials. PCEC is cheaper to produce. Elsewhere, purified vero cell vaccine (PVRV) (VerorabTM, Aventis Pasteur) is widely available, but the dose is a vial containing 0.5 mL. Experimental DNA-based vaccines have not yet been developed for use in man (Perrin & Jacob 2000).

Pre-exposure prophylaxis
( World Health Organization 1997; Centers for Disease Control 1999; Salisbury & Begg 1996). No rabies deaths have been reported in those given pre-exposure prophylaxis with post-exposure boosting. Pre-exposure immunization is indicated for all those at occupational risk of contact with a rabid animal or rabies virus, in quarantine facilities, customs departments, zoos, laboratories or hospitals, and for residents of or visitors to areas where dog rabies is endemic. Those staying in rural areas of foreign countries where rabies is enzootic in other mammals (foxes, jackals, wolves, coyotes, mongooses, bats, etc.) should seek advice about the risk.

Pregnancy is not a contraindication to rabies vaccination. Subsequent post-exposure treatment will be simplified and much cheaper after a prophylactic course. The high cost of vaccine is the only constraint to widespread pre-exposure immunization.

Pre-exposure vaccine regimens
One dose of HDCV, PCEC or PVRV is given i.m. into the deltoid on days 0, 7 and 28 (or 21). An economical but effective alternative is to give the vaccine intradermally i.d. if more than one person is to be immunized. The dose of 0.1 mL of any of these vaccines is injected i.d. over the deltoid to raise a papule. If the injection is too deep, withdraw the needle and repeat the procedure. Opened ampoules should be stored in the fridge and used the same day.

Chloroquine antimalarial chemoprophylaxis may inhibit the induction of rabies antibody after i.d. vaccination, so the larger dose must be given i.m. A booster dose by either route one year later enhances and prolongs the immune response, which lasts more than 10 years in 96% of people (i.m. vaccine was used in this study) (Strady et al. 1998). A booster dose or an antibody test should be performed six monthly for rabies laboratory staff and all those at continued high risk of infection. For other people, Strady recommends testing the neutralizing antibody level after three years to detect the ‘low responders’. If the titre is < 0.5 IU, booster doses should be given every three years, and if > 0.5 IU, boosters can be given every 10 years (Strady et al. 2000). If no serology is available, or affordable, boost every 5–10 years. In the USA, frequent serology and boosters are recommended only for those at high risk, but no boosters after the primary course for travelers (Centers for Disease Control 1999). The level of antibody may be lower following i.d. vaccine, but the quality of the secondary immune response to a booster dose is similar for i.d. and i.m. injections. Measurement of neutralizing antibody levels after treatment is necessary only if immunosuppression is suspected, or to determine whether a repeated booster dose is needed.

Post-exposure treatment

The decision to treat
Exposure to rabies infection is suggested by: Contamination of a wound or mucous membrane by an animal’s saliva. Intact skin is a barrier to infection. Bites on the head, neck or hands, or multiple bites carry a higher risk of infection.

Abnormal or altered behaviour of the biting animal such as partial paralysis or aggression are characteristic of rabies. Vaccination of domestic animals is not always protective. The local epidemiology of rabies will identify important local vectors (Warrell et al. 1995), for example dogs and cats in Asia, Africa and parts of Latin America; mongooses and jack-
als in Southern Africa; foxes and wolves in parts of Europe, and foxes, raccoons, skunks and coyotes in regions of North America (Smith 1996). Infected bats have been found in every continent except Asia. Other wild or domestic mammals may transmit rabies. Rabies-free areas include Sweden, Norway, Finland, Iceland, Ireland, Switzerland, Greece, Italy, Portugal, Mediterranean islands, Singapore, peninsular Malaysia, Hong Kong islands, Japan, Papua New Guinea, Taiwan, New Zealand, Antarctica, some Caribbean and Pacific islands, and the UK.

To confirm exposure, rabies antigen must be demonstrated in the animal’s brain.

If rabies exposure is suspected, post-exposure prophylaxis must be started as soon as possible. The greater the delay in starting treatment the greater the risk of virus entering a peripheral nerve, where it becomes inaccessible to immune attack. If in doubt vaccinate, it is worthwhile even if several weeks have elapsed.

**Post-exposure treatment for those without a previous course of vaccine**

This consists of three parts:

**Treatment of wounds**

Immediate washing, scrubbing and flushing with soap and water is recommended for all animal bite wounds, including those without risk of rabies. This treatment can be 50% effective in preventing rabies experimentally (Kaplan et al. 1962). Then apply either 70% ethanol or povidone iodine. Avoid or postpone suturing the wound. Tetanus prophylaxis may be needed. Consider giving a prophylactic antimicrobial agent for serious bites or those on the hands (coamoxyclav, doxycycline or erythromycin for dog or cat bites).

**Active immunization with vaccine**

A course of five i.m. injections of HDCV or PCEC vaccine are given into the deltoid on days 0, 3, 7, 14 and 28 (World Health Organization 1997; Centers for Disease Control 1999; Salisbury & Begg 1996). Although not officially recommended in the UK, economical i.d. post-exposure treatment has been used for 15 years in Asia (World Health Organization 1997) (see below).

**Passive immunization**

Rabies immune globulin (RIG) should be given with every primary post-exposure treatment. It is essential for severe exposure to infection: bites on the head, neck or hands or multiple bites. Passive immunization provides some protection for the 7-10 days before vaccine induced immunity appears. RIG apparently neutralizes virus in the wound and enhances the T lymphocyte response to rabies (Celis et al. 1985). The dose of 20 units/kg body weight of human RIG (or 40 units/kg equine RIG) should be infiltrated deep around the wound. If this is anatomically impossible, give the rest by i.m. injection at a site remote from the vaccine, but not into the gluteal region. For multiple bites the RIG can be diluted two- or three-fold in saline to ensure infiltration of all wounds. The recommended dose of RIG must not be exceeded as this will impair the immune response to the vaccine. Serum sickness has not been reported with human RIG. World-wide there are problems of production and supply of RIG, which might increase in the future, especially with the restrictions imposed by possible microbial and prion contamination. Novel strategies to manufacture immunoglobulins in vitro are being explored (Morimoto et al. 2001).

**Post-exposure treatment in previously vaccinated patients**

Treatment of animal bites is always urgent. Immediate wound cleaning is imperative. An abbreviated course of two doses of vaccine may be used, provided that a complete pre or post-exposure course of a tissue culture vaccine has been given, or a serum rabies neutralizing antibody level of > 0.5 IU/mL has been recorded previously. An i.m. dose of vaccine is injected into the deltoid on days 0 and 3. RIG treatment is not necessary (World Health Organization 1997; Centers for Disease Control 1999; Salisbury & Begg 1996).

**Efficacy of post-exposure treatment**

Indian studies have shown that the untreated mortality from proven rabid dog bites is 35–57% (Warrell et al. 1995). Optimal modern post-exposure treatment given on the day of the bite to healthy recipients is practically 100% effective. ‘Failures of treatment’ are fail-
Optimal modern post-exposure treatment given on the day of the bite to healthy recipients is practically 100% effective. ‘Failures of treatment’ are failure to deliver the three components correctly and promptly, or failure of the patient to mount an immune response. For example: if there is delay in starting treatment; failure to complete the course; injections of vaccine or RIG into the buttock; or immunosuppression by drugs, HIV, cirrhosis or other illness. Only two people are known to have died despite complete prompt treatment with modern products (Hemachudha et al. 1999).

Efficacy against rabies-related viruses (see above)
Studies have given varying results but HDCV does afford some protection against European bat lyssaviruses (genotypes 5 and 6) and Australian bat lyssavirus (genotype 7), but is less effective against Duvenhage (lyssavirus genotype 4) and gives little or no protection against Mokola virus (genotype 3) (King & Turner 1993; Badrane et al. 2001).

To overcome immunosupression or antigenic incompatibility, the antigenic stimulus can be increased by doubling the initial dose of i.m. vaccine or by dividing the single dose between multiple sites intradermally (see below).

Adverse effects of tissue culture vaccines
There is very wide variation in the incidence of side-effects. Mild erythema and pain at injection sites are reported in 7–64% of HDCV recipients, and local irritation is more common (13–92%) after i.d. treatment. Generalized symptoms of headache, malaise, and fever occur in 3–14% (World Health Organization 1997), and up to 3% reported a ‘rash’ distant from the injection site. There are data similar for PCEC and PVRV vaccine. Very rare neurological illness has been reported (see survival after rabies, above). A systemic allergic reaction 3–13 days following late booster injections of HDCV, has been observed in 6% of those vaccinated in the USA, however, reaction rates were not evenly distributed between the groups of volunteers (Dreesen et al. 1986). The urticarial rash, angioedema and arthralgia responds to symptomatic therapy. It is possibly caused by an IgE mediated reaction to -propiolactone-modified vaccine components (Warrington 1987). For people
at continued high risk of infection, measurement of antibody is advisable to confirm the need for repeated booster doses of vaccine.

What to do if bitten outside the UK
(World Health Organization 1997). If bitten by a mammal in a rabies endemic country (or if any direct contact with a bat in the Americas), immediately wash the wound thoroughly (see above). Seek advice on the risk of rabies infection from a local doctor. If post-exposure treatment is indicated, find one of the mentioned tissue culture vaccines (and for primary treatment, RIG) urgently. If only locally produced animal brain vaccine is available (e.g. Semple or suckling mouse brain vaccines), begin treatment despite the risk of allergic encephalomyelitis, and change to one of these European vaccines as soon as possible. If RIG is not available, it can still be given up to seven days after starting vaccine. Equine RIG is widely used in Asia and Africa, and there is a 1–6% incidence of serum sickness, but anaphylaxis is rare. If the risk of rabies exposure seems high it is worth curtailing the holiday to seek a recommended vaccine and RIG.

Two other post-exposure treatment regimens are recommended by the WHO (World Health Organization 1997). They are economically multi-site intradermal regimens, which use only 40% of the amount of vaccine needed for the standard i.m. course. The eight-site regimen gives an accelerated antibody response: on day 0 use a whole 1 mL vial to inject 0.1 mL PCECV or HDCV i.d. at eight sites (deltoids, thighs, suprascapular, lower anterior or abdominal wall); on day 7 give 0.1 mL i.d. at four sites (deltoids, thighs) and on days 28 and 91, 0.1 mL i.d. at one site (Warrell 1985). There is no report of the use of PVRV, which contains 0.5 mL per ampoule, with this schedule, but the equivalent dose would be 0.05 mL per site.

The two-site intradermal post-exposure regimen has been used widely in Asia accompanied by RIG, or in those who have had previous rabies vaccine. It was designed for use with PVRV (Chutivongse et al. 1990), with an i.d. dose of 0.1 mL per i.d. site. If the other, 1.0 mL, vaccines are used, each i.d. dose must be 0.2 mL. On days 0, 3 and 7, give one i.d. dose at two sites (deltoids); on days 28 and 91 give one i.d. dose at one site (deltoid)

The two i.d. regimens use the same amount of vaccine, and aseptic techniques are essential when sharing ampoules. Opened ampoules should be used the same day. A comparative study showed that the eight-site method induces neutralizing antibody more rapidly and to higher levels than the two-site regimen, which is especially important when RIG is not available (Madhusudana et al. 2001).

SUMMARY OF PRACTICAL POINTS
• A history of travel during the previous year or more is needed for all encephalitis patients.
• The methods of diagnosing rabies encephalitis during life are rapid and sensitive.
• Rabies encephalitis is incurable, and there are no reported survivors of furious rabies.
• Vaccines are safe and effective. A course of pre-exposure prophylaxis for those at risk is three injections, and a booster after a year enhances and prolongs the antibody response. Cost is the only contraindication to treatment.
• Post-exposure treatment is urgent. Clean the wound thoroughly, assess the risk of rabies infection and give vaccine and RIG immediately if indicated.
• If previous immunization is certain, only two doses of vaccine and no RIG are needed post-exposure.

REFERENCES


