RABIES

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PART I

Definition

Rabies (Rage, Tollwut, Rabbia) is an acute infectious viral disease of the central nervous system (C.N.S.), ordinarily transmitted by bite from animal to animal, and from animal to man. It is characterised by abnormal behaviour or madness and is almost always fatal.

The disease as it occurs in man is also called hydrophobia because it commonly results in episodes of painful spasmodic contractions of throat muscles following attempts to swallow water (Johnson 1970).

Notes: (a) Hydrophobia does not occur in animals—dogs keep on drinking. (b) Other diseases resulting from animal bites include pasteurellosis from cats and dogs: B. virus from monkey bites, tularaemia, rat-bite fever, tetanus (Benenson 1970).

Introduction

There is currently a widespread epidemic of wild life Rabies extending from the Arctic Circle to the Tropics in both the New World and the Old World.

There was a similar outbreak about 100 years ago—the possibility of a Rabies Cycle has been mooted (West 1972). Indeed the concept that rabies survives in silent epidemics in maintenance hosts is very attractive. The existence of a widespread silent reservoir would be the most vital factor for the survival of the rabies virus, rather than the relatively unimportant but more obvious transmitters such as dogs and foxes.

Britain has been free from rabies so long that 4 cases in dogs in or out of quarantine in 1969/1970 came as a great shock to the Government, the general public and the general practitioners alike. As a consequence of these very worrying incidents the 'Whitehouse' Committee set up by the United Kingdom (U.K.) Government issued a detailed report which is compulsory reading for everyone with interest in this subject;

"In many parts of the world rabies remains one of man's most feared diseases, causing numerous deaths every year; and its victims often die horribly, in great pain and distress.

Rabies is also a serious threat to animal life. It occurs naturally in many species in the wild, and it is highly probable that all mammals are susceptible.

If an outbreak of rabies were to occur in this country or if it were to become established in wild life here, the cost in terms of danger and fear, personal restrictions and destruction of wild life would be considerable, quite apart from the substantial public and private expenditure which would be called for" (Report of the Committee of Inquiry on Rabies 1971). "It is unfortunate that rabies tends to be classed among the esoterica of medicine until it strikes uncomfortably close to home" (Brit. med. J. 1969).

Enzootology: Epidemiology

The great question as to how and why the rabies virus has failed to get established in wild life in the British Isles remains unanswered (Hole 1969). In Europe an epizootic of wild-life rabies starting from Central and Eastern countries during World War II has spread westwards in our generation at the rate of 40 kilometres per year. The Rabies time-table for Europe is:—

Crossed the Elbe 1950. Reached the Rhine 1960. Denmark 1964. Belgium, Luxembourg and Austria 1966. Switzerland 1967 and France 1968.

The fox is the culprit in Europe, and sophisticated modern methods such as Radio-telemetry are being used to study fox ecology and rabies epidemiology; foxes are trapped and a radio-transmitter is strapped around their necks, then the animals are let free, only to be followed at a discreet distance by mobile field tracking units equipped with radio receivers which monitor by triangulation methods the animal's movements (see *World Medicine*, 16 June 1971).

It is thought that when more than half the fox population is killed by rabies, the disease burns itself out in that particular region, but within three years the area is repopulated once more by foxes and rabies re-appears once more. It is possible that in some foxes inapparent infection develops without any signs, such foxes could then well constitute a reservoir of rabies virus in nature (*Surveillance and Control of Rabies* 1969).

There are many other animal reservoirs of rabies (mostly *Mustelidae* and *Viverridae*) in various countries and different continents, such as for example skunks and racoons in North America, dogs in Latin America, wolves in Eastern Europe, meerkats in South Africa, jackals in North Africa, jackals, mongooses and monkeys in India (Irvin 1970).

Then there are the Chiroptera—vampires, insectivorous and frugiferous bats. The classical description of Bat Rabies by Hurst and Pawan (1968), which has been reprinted on more than one occasion is worth reading especially for its historic and medical interest.

As far as Great Britain is concerned, the following are possible routes of invasion:—

(a) The modern way of transporting goods in standard containers, which are packed and loaded in one European country and transported swiftly and directly to their final destination in the heart of another country, may well provide shelter, lodging and an unobtrusive means of transport for a rabid animal from across the Channel, which has been in the past a strong anti-invasion ditch. The possibility of the proposed Channel Tunnel offering another more direct threat, by its link to the continent where rabies has been endemic since time immemorial, has to be taken into consideration by its planners and constructors.

(b) It is not inconceivable that sooner or later, that flying mammal the Bat may wing its silent way from the mainland on to our European offshore island.

(c) As had happened in the notorious outbreak at the end of World War 1 (which resulted in rabies infection of 312 dogs, 8 cattle, 2 sheep, 3 pigs and 3 horses) some misguided pet owner may attempt to smuggle an animal into Great Britain to avoid the expenses of quarantine or the separation from a loved one.

(d) Another possible danger is that pets on board ship, berthed conveniently alongside quays or in harbours, may escape their owner's control, something which could occur all too easily once the symptoms of rabies affect an animal.

Virology

During the past few years the use of modern virologic techniques has led to a rapid progress in our fundamental understanding of the rabies virion itself and as applied to the biological, epidemiological and prophylactic problems of rabies.

The virus particle is best described as being bullet-shaped with one rounded end, and one flat end having what appears to be a depression or hollow like an arrow's notch. Rabies virus statistics are:—(a) Length—180 nanometres (n.m.). (b) Diameter—80n.m. (c) Surface, studded with spikes—6 n.m. (d) Internal—single-stranded ribonucleic acid helical nucleocapsid.

The virion's outer surface consists of a double-layered envelope which bears the spikes, the latter having knobs at their free projecting end. The spikes are made of glycoprotein, the antigen that induces in infected and vaccinated animals the serum neutralising antibodies so vital for immunologic protection. A similar cause and effect is attributed to the spike haemagglutinin of influenza viruses.

The virus can be grown quite successfully in a variety of tissue cell cultures; it is stated to grow better at 31° to 33° centigrade than at 37° centigrade, and growth may be aided by treating the host cells with ultraviolet light or Actinomycin D.

By electron microscopy the virion can be seen: (a) In matrices replacing cytoplasmic structures. (b) In cytoplasmic vacuoles. (c) Budding from cell surface.

The virions are arranged single or in closely packed arrays which tend to form a geometric pattern; the virus particles are more numerous inside the host cell than in the culture fluid outside, which suggests that on replication intracytoplasmically many virions are not released freely into surrounding environment.

In actual life however it has been shown that though in neurones the virus has an exclusively intracytoplasmic maturation, the mode of maturation of rabies virus in salivary glands effectively results in the release of virions from the marginal membranes of acinar cells in numbers sufficient for transmission via the animal's bite, which is the natural mechanism for survival in nature of the Rabies virus (Smith, Lauffer and Bang 1970, Dierks, Murphy and Harrison 1969).

Notes: Strains of rabies virus. "Two viruses isolated in Nigeria (Lagos Bat and Ibadan Shrew virus, also called Mokola) are serologically closely related to Rabies virus, but the relation of rabies-related viruses to the occurrence of enzootic rabies in wild carnivores is unknown.

Since 1969, workers in Czechoslovakia, West Germany and Switzerland have made 36 isolations from field rodents of viruses serologically related to rabies. There is no evidence relating the presence of these viruses in a small proportion of wild rodents (less than 2 per cent) to enzootic rabies in foxes" (Lancet 1973, Tignor and Shope 1972, Schneider and Schnoop 1972).

In the United States diagnosis of rabies in members of the order Rodentia (and the closely allied Lagomorpha) has been in the past over-estimated when based on the finding

of inclusion bodies. Re-appraisal of diagnosis by modern methods (Fluorescent Antibody Test (FAT), Mouse Innoculation (MI), Serum Neutralising Antibody (SNA)—q.v.) has revealed that these animals play only a minor part in the rabies problem affecting that country—4 to 5 rodents being truly rabies-positive per year (Winkler 1972).

Routes of infection

Innoculation. Rabies virus is usually transmitted by the bite of an infected animal. Infection cannot take place through unbroken skin but it is possible through uninjured mucous membranes. It is possible to infect biting arthropods experimentally but it is not known if these play any part in natural wild life rabies; the virus has been demonstrated in ticks (Ritossa and D'Agostino 1967). Viruses having same group antigen (nucleo-protein) as the classical rabies virus have been recovered from *Culicoides* in Nigeria (Kotonkan Virus-Kot) and *Mansonia* in the Sudan (Obodkiang Virus-Obo).

Inhalation. This has been dramatically but most unfortunately demonstrated by the deaths of two men who entered the Frio Caves in Texas, these caves sheltered an immense number of rabies infected bats. Subsequent experiments conducted on caged animals proved still further the actuality of respiratory infections (Constantine 1967).

Note: Presumably therefore bats can become infected either by fighting and biting each other, or by inhalation of rabies virus particles. The rabies virus is also found in the excreta of animals and it has been surmised that foxes get infected by this method 'when nosing the tail'.

Ingestion. The rabies virus is not only excreted in the urine as stated above, but it can also be secreted in milk, thus infected mothers may infect their suckling young. On the other side of the picture it has been shown that mother mice may be infected by cannibalising their infected offspring (Fischman and Ward 1968).

It is possible that foxes, racoons, skunks, opossums, feral cats, and others, may eat stricken and fallen bats or other infected animals; moreover it is also possible that bats may get infected by eating insects that feed as larvae on carcasses of rabid animals (Behbehani 1972, Correa-Giron, Allen and Sulkin 1970).

Pathophysiology

Rabies is a rather peculiar disease among the communicable diseases in that the pathogenesis involves:

(a) Spread from its site of inoculation centripetally via the nerves. Though the virus may be present in the blood under some circumstances the consensus of opinion is that viraemia does not usually occur nor does it play a significant role in the pathogenesis of rabies—contrast this with the Poxvirus or the Enterovirus (Christie 1969).

(b) The rabies virus multiplies in the neurones of the grey matter and spreads centrifugally again via the neural routes so that it can be detected peripherally in the Salivary Glands, Lachrymal Glands, Nasal Mucosa, Kidney, Pancreas, Adrenal Gland.

“Studies on the pathogenesis of rabies suggest that acute rabies infection is composed of three phases: (a) Extraneural phase without demonstrable multiplication of virus. (b) Intraneural progression of infection within the central nervous system. (c) Dissemination of the virus via neural pathways” (Schneider and Schnoop 1972).

"Although it is possible for the virus to invade the C.N.S. directly from the bite wound, the fact that the virus can multiply in muscle tissue suggests that infection of this tissue is perhaps the more common primary route of infection with subsequent invasion of the C.N.S. via the neuromuscular pathways. Following intramuscular inoculation with rabies virus, an administration of an adrenocorticotrophic hormone can reactivate a latent infection; this indicates that the virus can infect and remain localised in muscle tissue" (Johnson 1969).

Note: "It seems that in vivo the inoculated virus can develop only in the neural filaments, the nerves, or the neural trunks" (Baltazard 1970).

The findings at postmortem are:

(a) *Macroscopically*—nothing very characteristic. Congestion of brain and meninges. Some cerebral oedema, resulting in flattening of cerebral convolutions.

(b) *Microscopically*—the earlier changes in nerve cells are ballooning, later pyknosis, karyorrhexis or even neuronophagia. Perivascular cellular infiltration. Focal and diffuse gliosis. Neuronal degenerative changes in spinal cord may be found in the posterior horns corresponding to area of bite or wound. A variable degree of demyelination may be seen (Bisseru 1972).

Note: "Neurohistology of fatal cases of rabies suggest that damage to the C.N.S. is often milder than after other viral encephalitides in which recovery occurs, for example, polio" Turner 1972).

In addition to the histological features, noted above it has been suggested that humoral allergy reactions may play a significant part in the pathogenesis of rabies. Thus while such antibodies as the Serum Neutralizing Antibody (SNA) are considered beneficial to the patient, and it is on this premise that early prophylactic treatment with Rabies hyperimmune sera is advised, other antibodies such as the cytotoxic or cytolytic antibody, may actually do the patient harm, no doubt in a well-meaning effort to burst open the cells in which the virus is replicating and thus expose the virions lurking within to the full effects of the SNA (Wiktor 1971).

"It has been shown that infection of cells with viruses that do not produce cytopathology (rabies) results in the appearance of new antigens on the surface of the infected cells and that the interaction of specific antiviral antibody and complement with these antigens can damage the cells" observed Allison 1972. This same worker puts forward the interesting hypothesis that the mononuclear cells seen in the perivascular cuffs so beloved of neurohistologists reporting on viral encephalitides are cells which migrating out of the blood vessels will then apply themselves to the neurones in which the virus is replicating—they suggest that these cells have a cytotoxic potential which merits further study and propose the name A cells (*cf* T and B lymphocytes) for these cells (effector cells involved in antibody-dependent cell-mediated cytotoxicity).

The virus genome may persist in latent state in an infected cell until triggered off to reassume pathogenicity by, for example, a stress condition. The symptomatology that follows is then dependent on the relative excitation, or depression, of the C.N.S. infected focus.

The current European epidemic strain of rabies appears in dog's saliva coincidentally with rapid signs in animals but may be present for up to three days in the saliva before signs of abnormal behaviour appear in the suspect animal.

The incubation period of rabies in man varies quite a lot, generally speaking it may be summarised as follows:—

Rabies incubation period time-table—(a) 40 per cent of patients—31 to 60 days. (b) 30 per cent of patients—less than 30 days. (c) 30 per cent of patients—more than 60 days.

Note: Is this a record! “ A woman aged 49 years had been bitten on the right leg by a rabid dog and had an incubation period of no less than nineteen and a half years. Investigations showed that no case of human or animal rabies had occurred in the area from which the patient came for at least ten years (Gaurila, Iurasog and Luca 1967).

Signs and symptoms

After an incubation period varying from three weeks to months or even years, a rabies victim will show—Local paraesthesias. Acute anxiety. Fever. Tachycardia. Thirst. Throat spasms. Hydrophobia. Dyspnoea. Priapism in males. Convulsions. Paralysis. Drowsiness. Coma or Death. The causes of death are (a) Rabies encephalitis. (b) Rabies myocarditis. (c) Rabies pneumonitis.

“ The patient with rabies is usually mentally alert for a week or more after the onset of his first symptoms, during which a variety of alarming problems develop. In untreated cases death usually occurs from cardiac or respiratory arrest four to eight days after onset in a patient who is acutely aware of every stage in the process of his disintegration. If however, this early death is forestalled by intensive medical support the patient passes into a phase of coma that holds no special terrors. Much of the agony of human rabies is a result of a combined effect of three factors—(a) A disease with frightening symptoms. (b) A mentally alert patient. (c) An environment that communicates despair ” (Hattwick 1972).

Diagnosis

A clinical awareness and a careful history taking are most important in making a diagnosis of this dreaded disease.

“ The laboratory occupies a central place in meeting the threat of rabies because upon its verdict often depend both the decision whether or not to proceed with a long and painful course of treatment and the decision on the need to institute elaborate measures for controlling an epizootic in a community ” (Kaplan 1973).

The diagnosis of rabies depends on:—

(i) *Demonstration/Isolation of Rabies virus.* (a) Histopathologic methods. (b) Fluorescent antibody. (c) Mouse inoculation. (d) Immuno-precipitation. (e) Complement-fixation. (f) Tissue cell culture. (g) Egg culture. (h) Electron microscopy.

(ii) *Demonstration/Estimation of Rabies antibodies.* (a) Fluorescent antibody. (b) Serum neutralisation. (c) Haemagglutination inhibition. (d) Immuno-precipitation (Gel diffusion). (e) Complement-fixation. (f) Immuno-cytolysis. (g) Passive haemagglutination. (h) Rabies virus interference—inhibition. (j) Radio-immuno assay.

(iii) *Non-specific tests.* (a) White blood cell count—the total cell count is increased and may reach 20,000 to 30,000 with polymorphonuclear leucocytosis. (b) Urine—the following may be present—protein, casts, glucose, acetone, oliguria. (c) Cerebrospinal fluid—there may be increased pressure, increases in protein and in cells.

Note: Take specimens early in disease: Cerebro-spinal fluid (cfr mumps). Sputum—because virus is excreted via glands of eyes, nose and throat and lung parenchyma. Saliva—from direct openings of submaxillary salivary glands. Swabs—from nose and throat. Urine (cfr mumps). Stool (cfr enteroviruses). Paired sera, acute/convalescent, or even a series of sera (Johnson 1969).

Differential diagnosis

The following affections are to be kept in mind: Tetanus. Hysterical rabies. Post-vaccination complications. Viral meningo-encephalitides. Poison.

“In view of the possibility of confusion with rabies laboratory support for the diagnosis of allergic encephalomyelitis by the following tests should be sought: Delayed hypersensitivity skin tests. Lymphocyte transformation tests. Assays of serum antibodies against extraneous protein of vaccines, whole nervous tissue or myelin derivatives.

The most specific of these tests measure response to myelin derivative, called basic encephalitogenic protein, which is believed to be responsible for reactions to rabies vaccines in man” (Hattwick et al 1972).

Treatment and prophylaxis

For reasons which will vary with each individual case under review a confirmatory laboratory diagnosis may not always be had on a “results-while-you-wait” basis, though this indeed is the ideal aimed at in well-to-do countries with a sophisticated Community Health Department, Laboratory Service, Transport facilities and so on, and the clinician is often face to face with a cruel dilemma. This happened to me when members of my own family came in contact with a suspected animal.

“It is not hydrophobia but rabidphobia which constitutes the major and most troublesome problem to the practising physician and health officer” (Sellers 1948).

Synopsis of treatment

Patient (or parents, friends, helpers). Wash wound with soap and running water immediately. Time is vital. Vodka, Gin, Brandy are all good disinfectants.

Medical Practitioner. Wash wound sec. art.—thoroughness with gentleness is vital. Wounds are not to be sutured. Area Rabies Committee to be contacted for advice. Give serum systemically and infiltrate locally. Refer to Treatment Centre.

Treatment Centre. Anti-rabies serum, if not already given. Vaccine. Continuous monitoring, intensive care and aggressive treatment as indicated. Medical and Nursing personnel should take appropriate precautions against possible infection when looking after a rabies patient.

Post-exposure anti-rabies prophylaxis tabulated guide

A tabulated guide, modelled on the United States Public Health Service 1972, is given in Table I.

Dosage

See the manufacturer's leaflet which accompanies each batch of vaccine; the number of inoculations and the volume injected vary not only from country to country, but also from one manufacturer to another. The usual schedule of immunisation is 14 to 21 daily doses given subcutaneously, with booster doses on the 10th and 20th day (Atanasui

Table I
Post-exposure anti-rabies prophylactic tabulated guide

Animal		Trêatment	
Species	Condition	Mild exposure	Severe wounds
Domestic	Normal Abnormal	Watch animal Vaccine (Stop treatment if laboratory tests negative)	Serum. Watch animal Serum and vaccine
Wild	Any	Serum and vaccine	Serum and vaccine

et al 1967) after the primary course; a final booster dose on the 90th day ensures high levels of protective antibodies.

At the Pasteur Institute, Paris, a shortened vaccine regimen was introduced in 1968; this consists of 7 daily SCI of 2.5 ml followed by 2 intradermal doses of 0.25 ml on days 11 and 15. Serum antibody titres are reported to be superior to those obtained with usual vaccination programmes, while at the same time the volume of vaccine injected was reduced by 50 per cent (Lepine et al 1971).

As regards the booster doses it has been suggested that these should be spaced out at much longer intervals than has been generally advocated, and possibly more doses should be given as well; thus Habel (1966) was of the opinion that "a more effective regimen consists of booster doses of vaccine at 20 days, at 2 months, and 6 months after initial series".

Antiviral chemotherapy

"The search for antiviral chemotherapeutic and chemoprophylactic agents has been intensified in the past few years. The application of high-titering interferon and of interferon inducers has been remarkably successful in protecting laboratory animals against a number of viral diseases. Karl Habel was one of the first to describe the protective effect against rabies of an interferon inducer the polyinosinic/polycytidylic acid complex. I feel certain that an agent originating from one of these groups will soon form part of and thus enhance the efficacy of post-exposure rabies treatment" (Fenje 1971).

For the immediate future however, the present position has been well summed up by Hattwick who puts the case thus—

"although a number of experimental agents have been tried in clinical rabies, there are no data suggesting that any specific antirabies agent is of value once the clinical symptoms appear.

Human rabies immune globulin, beta phenylserine, cytosine arabinoside, polyribonosinic—polyribocytidylic acid, quinine, arsphenamine have all been proposed and tried in treatment of clinical rabies without documented success.

Since no specific antiviral agent is known to be effective once symptoms have been developed, the treatment of clinical rabies must rely on aggressive supporting care—to prevent hypoxia, intracranial hypertension, cardiac arrhythmia, siezures, superinfection.

We know that such care can cure" (Hattwick et al 1972).

The treatment of rabies reminds me in some ways of the use of vaccinia virus to vaccinate the immediate contacts of a smallpox patient in an urgent effort (a race against time really) to establish immunity and so give protection to the presumed infected vaccinees before the variola virus matures, replicates and gets disseminated during its fortunately longer incubation period.

"The management of patients exposed to a risk of rabies is aimed at producing an immune state as quickly as possible so that the virus will not become established in a peripheral nerve along which it will travel to the central nervous system. Rabies anti-serum is administered to try to localise the infection to the wound and to establish immediate immunity.

Vaccine is then given frequently and at a high dosage so as to establish the maximum active immunity in the shortest possible time" (Brit. med. J. 1970).

Pre-exposure prophylaxis

"Prophylactic immunisation in man can be achieved with Nervous Tissue Vaccine (NTV), High Egg Passage of Chicken Embryo Origin (HEP-CEO) or Duck Embryo Vaccine (DEV). The DEV is most used in this country and in the United States of America (U.S.A.) because it is readily available and because of the lesser risk of side effects than with NTV" (Report of the Committee of Inquiry on Rabies 1971).

"The vaccination schedule consists of two doses (1.0 ml DEV SCI) given six weeks apart, followed by a reinforcing dose six months later. A reinforcing dose should be given annually. A blood test is taken after the third injection and after all subsequent injections to ensure that satisfactory protection against rabies has developed" (Memo Tay 37565 1971).

"DEV given in intradermal doses (0.2 ml) either weekly or monthly (three doses) followed by a booster 10 months later also gives good results (77 per cent and 81 per cent seropositivity respectively in vaccinees)" (Kaplan 1970).

In North America rabies vaccine of tissue culture origin has been used with good effect—one recommended schedule consists of 3 injections of 1 ml IMI given at monthly intervals, followed by boosters of the same volume 3 to 6 months later and annually for those at continuous risk.

As far as I am aware not a single death has been recorded in persons who have so far taken advantage of pre-exposure prophylaxis. A person so immunised (as monitored by adequate serum neutralisation antibody titre) if subsequently requiring treatment may be dealt with as follows:?

(a) Mild exposure: One dose of potent vaccine. (b) Severe exposure: Five daily doses, plus a booster 20 days later (W.H.O. Techn. Rep. Ser. 321/1965).

A person who has failed to develop antibodies, or whose neutralising antibody titre has not been determined, should receive a full course of anti-rabies treatment when exposed.

Conclusion

At Home

"Veterinary surgeons need to be constantly aware of the possibility that a case may appear in their surgeries.

Doctors would be well advised to keep themselves informed about the disease and make sure that they have the rules for postinfection prophylaxis at hand.

Enough vaccine and human antirabies serum should always be available to treat a substantial number of people at short notice" (Brit. med. J. 1972).

Abroad

"A person who has been bitten or scratched by an animal while abroad should consult a local doctor immediately.

If the owner or person in charge of the animal is known, travellers are advised to take the name and address, and to give their own name and address in return, so that information about the animal, including the results of any laboratory tests, can be readily transmitted and received.

In the case of a domestic animal, it is also useful to know whether it has been vaccinated against rabies, and if possible when and where, since this would considerably reduce the risk of infection" (Dept. of Health and Soc. Sec. 1972).

At home and abroad

It is desirable to have a Rabies Standing Committee in appropriate regional areas, to deal with each individual case of animal bite injuries; such a Committee is to be constituted of the following members:—

(a) Veterinary surgeon. (b) Physician. (c) Communicable disease expert (Medical Officer of Health). (d) Virologist. (e) Public relations officer.

PART II

Demonstration/Isolation of rabies virus

Histopathologic methods

(a) The direct microscopic examination of smears and impressions, taken from various areas of the brain, and stained with Seller's stain is one of the easiest, quickest and commonest test employed.

The presence of intracytoplasmic inclusion bodies with typical smaller particles in their interior—the Negri Bodies—is diagnostic of rabies. This test takes only 30 minutes in all.

(b) Tissue material either processed by the frozen section technique or fixed in Zenker's fluid and stained with Eosin and Methylene Blue, Mann's, Lepine's, and others.

(c) The introduction into histochemistry of enzyme-labelled antibodies for the localisation of antigens has been developed into a diagnostic test for the detection of rabies antigen in smears or frozen section by means of the Direct and Indirect Immunoperoxidase staining techniques.

Histological methods are useful when permanent mounts are desired for record or research purposes.

Suitable areas of the brain for examination are: Hippocampus (Ammon's Horn), Thalamus, Pons, Medulla, Cerebellum, Frontal Cortex, Parietal Cortex, Spinal Ganglia are also useful.

Note: Eight slides are prepared by impression or smear from each area. This provides four slides for histopathological studies and four for FAT staining (q.v.).

Fluorescent antibody tests

(a) The direct fluorescent antibody test is the most important rapid diagnostic test for rabies in universal use. Diagnosis is possible within 6 to 8 hours. It is particularly valuable when traumatic/degenerative changes in brain substance make examination for Negri bodies difficult or impossible.

(b) Frozen sections: Skin biopsy. American workers have found that (at p.m.) frozen tissue cross-sections of salivary glands, lachrymal glands and cornea give a good easy to read definitive fluorescent picture (Martell and Aldasoro 1972).

It has been observed that rabies virus antigen may be detected in the skin by FAT in appreciable time (1 to 4 days) before the onset of clinical signs and therefore skin biopsy has been proposed, the upper lip from larger animals appears to be the ideal site to biopsy because of its rich nerve network surrounding tactile hairs (Smith et al 1972).

(c) The Intra-Vitam Corneal Impression Test, done on living patients, both animal and human has been put forward by German workers at Tübingen. It has been used successfully in at least 3 human patients; and no doubt more advantage of this method will be taken in the future. It is claimed that in most cases the viral antigen can be demonstrated in the cornea before it appears in the salivary gland (Schneider 1969).

Note: A recently reported accelerated method of doing FAT shortens the time required for the diagnosis of rabies by immuno-fluorescence to less than one hour (Larghi and Siméñez 1971). It is no longer necessary to keep a suspect animal alive as long as possible. The FAT will pick up the virus antigen where histopathological methods fail to detect Negri bodies, and when even the Mouse Inoculation Test is negative because of non-infectiousness of the virus or because of low pathogenicity of some strains (Lennette and Emmons 1971).

Mouse Inoculation

This 'biological' test is done by inoculating white laboratory mice intracerebrally with a suspension of infected material. Mice dying between 5th to 30th day after inoculation are presumed to have had rabies—but the diagnosis must be confirmed by the demonstration of Negri bodies (Fig. 1) or a positive FAT.

The Mouse Inoculation Test is a valuable test and is done in all cases, however the time taken to do the test takes too long if treatment is to be given to the patient. Therefore for practical diagnostic purposes one or two mice are sacrificed daily from the 5th day onwards, or even earlier (Bagnaroli, Larghi and Marchevsky 1970).

Immuno-precipitation

The Gel Diffusion Tests are sensitive tests for either rabies antigen or rabies antibodies. The technique consists in punching out cups in agar gel, and filling some of the cups with suspect infected material which is allowed to diffuse and combine with known potent specific rabies anti-serum placed in other adjacent cups. In positive tests precipitation lines are formed, easily visible to the naked eye.

Complement-fixation

The complement-fixation method can be utilised in the laboratory diagnosis of rabies using the suspect test material as antigen. In some cases, where the brain tissue,

(Courtesy of Dr. Frederick A. Murphy, C.D.C., Atlanta)

Fig. 1. Negri body. Human isolate of rabies virus in mouse neuron. Bullet shaped virus particles lie between smoothly granular condensed viral nucleocapsid inclusion material (X 35000).

or the salivary gland tissue, was putrid or degenerated thus preventing easier tests, the complement-fixation test has been of some value.

It has been claimed by Japanese workers that rabies antigen can be demonstrated by complement-fixation test in the brain of animals infected with 'weak' strains long after the rabies virus itself has disappeared and have suggested that the complement-fixation test could be useful therefore as a specific and sensitive means for diagnosis of naturally infected animals (Ishii 1971).

Cell culture

(a) Progress in Rabies Research leaped ahead when attempts to culture the virus on tissue cells in the laboratory proved possible and successful. The virus can be grown on Hamster Kidney Cells (BHK-21) and Human Foetal Diploid Cells; it can also be shown to produce Cytopathic effect (CPE) on Green Monkey Kidney Cells. Tissue cell culture is not useful for practical diagnosis in clinical cases.

(b) In Vitro 'self' culture: It has been suggested that in vitro culture of suspected infected tissue cells obtained from the patient may hold particular promise to document possible recovery from rabies, or to detect latent rabies (Lennette and Emmons 1971).

Egg culture

In common with other viruses the rabies virus infects chick embryos by the yolk sac, allantoic sac and chorio-allantoic membrane route of inoculation.

The inactivated DEV for human prophylaxis and the live HEP/LEP-CEO vaccines for animals are examples of the successful 'avianisation' of the rabies virus. The method is not suitable for practical diagnosis of clinical cases.

Electron microscopy

As far as rabies is concerned, electron microscopy, so useful in smallpox diagnosis, is used only for study and research purposes; it may be possible to find a place in diagnosis as experience with it is gained.

Demonstration/Estimation of rabies antibodies

These tests may be used (a) In some instances for diagnosis of overt disease in man prior to death (or? recovery). (b) To assay the immune status of an individual after vaccination.

At least five antibody types may be found: SNA, CFT, Haemagglutination Inhibition (HAI), Pgt, Cytolytic.

Fluorescent antibody

The indirect fluorescent antibody test (IFAT) is carried out by layering a volume of test serum in normal mouse brain suspension, and an equal volume in rabies infected mouse brain suspension, over a preparation of rabies virus, then washing these preparations and finally staining them with fluorescein-conjugated anti-human globulin.

A positive fluorescent test is given by test serum if antibodies are present, while the other volume of serum acts as a control and should be negative as any rabies antibodies would be absorbed and neutralised by being treated beforehand with the rabies infected mouse brain.

Serum neutralising antibody

The presence of SNA can be detected and titre estimated by several techniques.

(a) *Mouse SNA*. Graded dilutions of virus are mixed with patient's serum, or graded dilutions of test serum are mixed with a constant dose of rabies virus, and inoculated intracerebrally into mice as described above.

(b) *Tissue cells*. Graded dilutions of test serum are mixed with a constant dose of virus and inoculated into a suitable cell system, for example, Grivet Monkey Kidney Cells. The titre is noted at which the CPE is neutralised.

(c) *Plaque reduction*. Strains of rabies virus produce 'plaques' in cell culture systems. Graded dilutions of rabies virus in test serum, and the same dilutions in normal serum is parallel, are inoculated in 0.1 ml amounts on to cell cultures of Baby Hamster Kidney Cells (BHK) suspended in agarose. The number of plaques is counted after 6 to 7 days incubation.

(d) *Fluorescent foci*. The inhibition of Immuno-Fluorescent Foci Formation. This new test is stated to be both rapid and accurate. Cultures of BHK cells are lawned in glass microscope slides to form a confluent monolayer of cells. Graded dilutions of test serum are mixed with equal volumes of rabies virus (Flury stain) and then each serum-virus mixture is inoculated on to the cell monolayer. The slides are incubated for four days and then stained by the FAT technique, the number of rabies infected cell foci are recorded. I am informed by Dr. George M. Baer that he and his associates at Laurenceville, Georgia have devised a Rapid Fluorescent Focus Inhibition Test (RFFIT) requiring only 24 hours for its completion.

(e) *Brain tissue*. It appears that infection stimulates cerebral antibody formation, which can be thus titred, for example, by the mouse inoculation test as noted previously,

When the brain tissue neutralising titre is but a small fraction of the serum titre, this suggests post-vaccination antibodies; when however the brain titres are high and comparable to the serum titres, infection with rabies virus is implied.

This test may be useful for the differentiation of 'recovered' patients from those who have been vaccinated or in a prodromal stage of infection (Kubes and Gallia 1943).

In this technique graded dilutions of test serum and virus antigen are incubated at room temperature in disposable 'micro-titre' plates. A suspension of goose red cells is then added and patterns of haemagglutination are observed after 40 to 50 minutes. This test requires further study before it is adopted for practical routine use (Halonen et al 1968).

Immuno-precipitation

The techniques of immuno-precipitation (Gel diffusion) is the same as that described above for the detection of test rabies antigen; here a known positive antigen is allowed to diffuse in agar jelly against the unknown test serum. Appropriate controls are incorporated in the test.

Complement-fixation

The complement-fixation test may be useful for diagnosis of rabies in cases where the patient has not been treated with serum or vaccines. The technique is that ordinarily used in routine CFT's; the CFT antigen is prepared from rabies-infected mouse brains.

Immuno-cytolysis

This test detects the cytolytic antibodies which appear in parallel to the neutralising antibodies. It has been shown that tissue cells in culture when infected by the rabies virus are lysed after exposure to rabies antibody and complement. This can be shown either by staining the culture with Trypan Blue or by the release of ^{51}Cr from labelled cells (Wiktor, Kuwert and Koprowski 1968).

Passive haemagglutination

In this test goose red cells are coated with the Challenge Virus Strain (CVS) antigen, and these sensitised cells are then utilised as passive carriers of the rabies antigen in simple cell agglutination tests to detect rabies antibodies. Results are stated to be better with this test than with CFT or HAI (Gough and Dierks 1971).

Interference-inhibition

The Rabies Virus Interference-Inhibition Test is a practical application of the well-known general interference phenomenon by which a virus-infected cell resists a super-infection by another virus.

The patient's serum is first incubated with rabies virus, and this mixture is then used to infect chick-embryo fibroblasts. This cell system is then challenged by another virus—the Semliki Forest Virus (SFV). If rabies antibodies are present in the patient's serum, the rabies virus is neutralised in the first stage of the test and it is therefore incapable of infecting the fibroblasts; consequently the non-infected cells are still receptive to infection by the SFV.

This is a somewhat more complicated test than the SNA tests described above; it has been proposed as a test to measure rabies antibodies after vaccination (Sziegoleit and Gerth 1971).

Radio-immuno assay

The W.H.O. International Conference Centre for Rabies, Philadelphia, U.S.A. has tried out this procedure for the evaluation of specific rabies antibodies according to their binding capacity to rabies antigen labelled with ^{125}I . This method is stated to be the most sensitive test for the detection of rabies antibody.

Note: Detection and Titration of Rabies Antibodies can be done on Cerebro-Spinal Fluid as well as on serum. It has been observed that specific rabies antibodies develop to a high titre in human rabies encephalitis—such is not the case in postvaccination allergic reactions (encephalomyelitis).

Vaccines—active immunity: delayed

Nervous tissue vaccine (Mammalian)

Vaccines are prepared from nervous tissues (NTV) of animals such as rabbits, sheep and goats. This basic type of vaccine has been in use for many years, since the days of the pioneer the great Pasteur himself—examples of such vaccines are Fermi, Semple, Hempt preparations.

A later development of this series of NTV are vaccines prepared from the nervous tissues of suckling animals, which are stated to be deficient of the immuno-encephalitogenic material present in the earlier type of NTV; and thus causes no, or at least much less, allergic neurological complications which follow so frequently the use of the older NTV. This type of vaccine is prepared from young mice, rats, lambs and rabbits—it is also known as Fuenzalida's Vaccine and is very popular in Latin America, and also used in the Union of Soviet Socialist Republics. "The large number of immature animals required poses production problems and also increases the chance of adventitious viruses in the vaccine" (Turner 1969).

Physio-chemical methods of removing the encephalitogenic factors require expertise but has been attempted (Kaplan and Turner 1968).

Avianised vaccines

The rabies virus has been adapted to, is grown in and harvested from, the embryos of fertile eggs—examples are the attenuated LEP-CEO and HEP-CEO used for rabies prophylaxis in dogs and cattle respectively; and also the inactivated DEV which has become so popular in recent years in North America, and has tended to supplant the more dangerous NTV in some countries.

The DEV may not pack as big a punch as the NTV; SNA titres produced by the latter vaccines are usually higher. When faced with a strong indication for vaccine prophylaxis I personally prefer the NTV, at least up to now.

Note: This working rule may have to be reevaluated when the more immunogenic, and less reactogenic, vaccines are marketed (q.v.)

Tissue cell culture vaccines

The immediate future may well see the introduction of these 'in vitro' laboratory vaccines. The cells used may be Baby Hamster Kidney Cells or the Human Diploid Cell Cultures; aluminium phosphate may be added as adjuvant. It is hoped that 3 doses only, perhaps fewer, of this type of vaccine may well serve the purpose of the numerous inoculations required of the NTV or DEV series (Sikes et al 1971). While not licensed in Great Britain they have been used for pre-exposure prophylaxis in North America.

Sub-unit vaccines

These experimental vaccines may possibly be the ones that will be used in the more distant future. They require more technical manipulations in their production and hence would be more expensive to manufacture. In these vaccines viral sub-units or other products of virus synthesis are utilised instead of intact virions (Crick and Brown 1969).

“The advantage of having a vaccine which contains the coat component of the virus but which is free of viral nucleic acid and core protein is obvious, since the presence of the core proteins may partially suppress formation of antibodies by coat components, and the release of viral nucleic acid from inactivated vaccine and its subsequent integration in the genome, with all its consequences, is at least theoretically possible (Koprowski 1972).

Observations

Vaccines and interferon. Some workers investigated the attractive possibility that rabies vaccines also acted as inducers of interferon, and reporting upon their researches, they stated, “The demonstration that concentrated vaccine induces interferon offers a new possibility in prophylaxis namely a treatment that combines interferon induction and immunization. The former would offer immediate protection against natural exposure, the latter would offer long lasting immunity” (Wiktor, Postic and Koprowski 1972).

Vaccines and cell receptors. “Blocking of receptor sites might be concerned in the protection afforded by vaccine therapy. Firm evidence of such sites is not yet available but inhibitory studies (unpublished work) and the structure of the rabies virus suggest that such sites may exist” (Turner 1969).

Serum—passive immunity: immediate

None of the currently established vaccines if used alone can guarantee protection against a severe rabies exposure.

The additional use of antirabies serum in such cases is now an established practice. The combined treatment has however some disadvantages (a) Heterologous serum usually of equine origin gives rise to adverse local and general reactions; “with one exception, and in spite of the negative skin tests, all individuals who received serum developed reactions” (Mooser 1972). (b) The course of vaccine inoculations following co-administration of serum needs to be prolonged, and booster doses given, to offset interference, by the serum antibodies, with the rabies antigen—this leads to a vicious circle of more post-vaccination reactions.

Efforts to eliminate serum reactions by using gamma globulin of equine, or better still of human origin are being made. This however is a more expensive product and may be beyond the financial means of most prophylactic centres where large numbers of exposed persons are treated. Furthermore the interfering effect of homologous serum on vaccine may possibly be more prolonged and stronger than that of the heterologous serum (Abdussalam and Bogel 1970).

“The currently available sera are commercially supplied in the dried form which can be stored in ordinary refrigerators for years without any loss of potency. At least 40 IU per Kg of body weight should be injected in a single dose intramuscularly. The

patient should be questioned about sensitivity to horse serum and tested for such sensitivity by skin tests; the administration of antihistamines before and after administration of serum has been recommended" (Behbehani 1972).

As mentioned in previous paragraphs the serum should also be infiltrated locally; and if available the use of human antirabies immunoglobulin would be desirable.

Recent work suggests that an optimal protective compromise between active response, passive protection and interference is achieved with 20 IU/Kg of rabies immune globulin of human origin (Cabasso et al 1971).

Note: Serum has also been administered intraventricularly and intrathecally (Cali 1970, Lennette and Emmons 1971).

There is general agreement that serum administration should be given promptly and as early as possible, it should also precede vaccine inoculation—and many suggest that there should be a time lapse of 24 hours between serum administration and the first vaccine injection (MacRae 1973).

A group of authoritative workers however, in experimental work on rabbits using tissue culture vaccine, have shown that while serum may inhibit the action of the vaccine unless the latter was delayed for several days, the giving of the vaccine 12 to 24 hours before the serum may overcome to some extent the inhibitory effect that exists between these 2 agents. These workers suggest that the generally accepted and currently popular combined serum first followed by vaccine therapy routine may well need revision (Wiktor, Lemer and Koprowski 1971).

Immunoglobulin class

In the past, attention has been wholly focused on serum as a whole. A group of U.S.A. workers have shown that the most important class of immunoglobulin in rabies protection is the IgG fraction. They suggest that the present heavy multi-dose vaccine schedule which promotes IgM may be wrong in conception, since IgM does not leave the circulation, and unlike IgG, cannot get at the appropriate tissue site soon enough to affect the disease. They therefore propose that a vaccine dose should be given as early as possible after infection, then there should follow an interval of waiting until the IgG response begins, and only then should the treatment with vaccines be resumed (Rubin et al 1971.)

Notes: The attention of the diligent reader who has read so far is drawn to the Pamphlet "W.H.O. Techn. Rep. Ser. No. 523 of 1973", Expert Committee on Rabies, Sixth Report, which should be read at least once.

The following publications also merit perusal (a) The book in the W.H.O. Monograph Series entitled "Laboratory Techniques in Rabies", No. 23 of 1973, and (b) Annex 3 "Requirements for Rabies Vaccine" in W.H.O. Techn. Rep. Ser. No. 530 of 1973.

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The Cantlie Society

The Cantlie Society of Charing Cross Hospital is a medical service dining club which perpetuates the name of Sir James Cantlie who founded a number of medical units which later became the first units of the Royal Army Medical Corps, Territorial Army.

The club holds two dinners each year and membership is open to any doctor who either qualified or has worked at Charing Cross Hospital and who has served in Her Majesties Forces either regular or territorial.

The Autumn Dinner of the Society will be held on H.M.S. President in November 1974, and it is hoped that the Spring Dinner of 1975 will be held in the Tower of London.

Your enquiries should be addressed to Dr. K. A. Howlett, New Charing Cross Hospital, London, W.6 who will be pleased to give further details.