CHAPTER I

INTRODUCTION

Rabies is an acute viral encephalomyelitis which is usually fatal to domestic and wild animals as well as to human beings. The disease is caused by the rabies virus which is present in the saliva of infected animals. The virus is transferred to the other animals by fouling skin wound with spittle (1). After a bite, rabies virus travels the nervous pathway from the periphery to the central nervous system (2).

In 1882, Pasteur and his co-workers established the ultramicroscopic nature of the infectious agent of rabies and its maintenance by serial passage in animal brain (3). Mammalian neural tissue is capable of abundantly producing virus, but it is not a source from which intact virus can be readily isolated and purified. Nevertheles, most attempts at characterizing the virus from this source gave complex and confusing results. Reliable and detailed biological and physico-chemical analyses were possible in the late 1960S,when methods of propagating virus in non-neural cell cultures were adapted (4). Subsequent exploitation of large scale cell-culture methods provided substantial amounts of material from which the virus could be readily concentrated and purified.

Properties of the virus

Morphology

Rabies virus is a member of the family Rhabdoviridae (from the Greek word "rhabdos"-a rod). Under the electron microscope, the characteristic structure of rabies virus is cylindrical resembling a bullet (Fig.1), with one rounded and one planar end. The average dimensions are 180 by 75 nm. Regularly spaced projections, 6-7 nm long, each with a knoblike structure at the distal end, cover the surface of the virion. The envelope structures surround a cylindrical core which contains the helical ribonucleocapsid. The intact core has approximately 30-35 coils of a single-stranded ribonucleoprotein which form a cylinder measuring 50 by 165 nm. (4,5).

In addition to the typical virus particle, anomalously shaped virus particles such as bizarre elongated filaments and V-or Y-shaped forms can be seen in preparation sharvested from cell cultures late after infection with rabies virus. Short or truncated particles are also produced when the virus grows in cell culture or mouse brain. They do not replicate in the absence of infectious virus and they interfere specifically with virus production in cell culture.They are defective interfering particles (4).

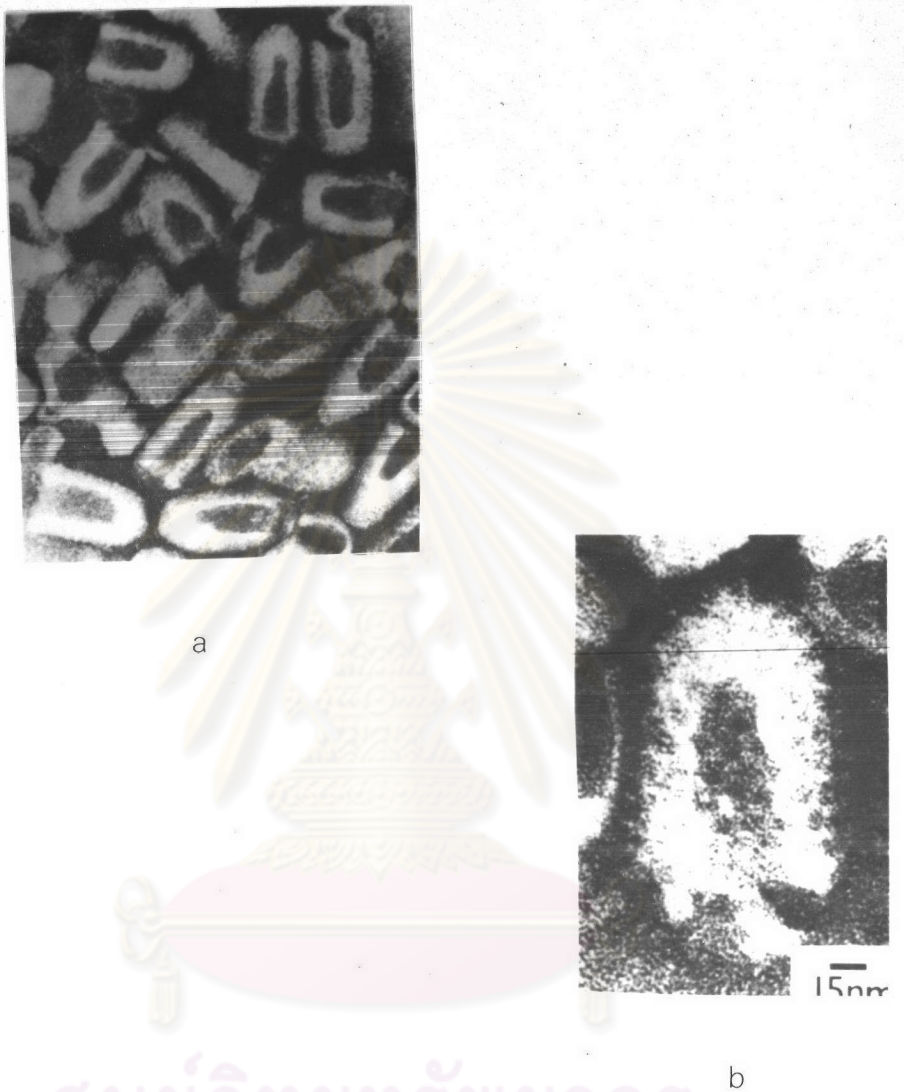

Figure 1. Electron micrograph of rabies virus showing a) the bullet-shaped b) the glycoprotein spikes and honeycomb structure.
(Photograph by G.J.Smale)

Biochemical constituents

The virus particle contains about 3.9 % RNA(6), 67 % protein, 26 % lipids (5) and 3 % carbohydrates covalently linked to lipid and protein (7). The viral nucleic acid isolated from purified nucleoprotein or from the intact virion has molecular weight of 4.5×10^6 daltons. It is sensitive to ribonuclease (6). The isolated RNA is not infectious (5).

The viral lipids consist of neutral lipids predominantly represented by cholesterol amounting to 4.4 %, other neutral lipids adding to about 1 %, phospholipids accounting for 11 % and glycolipids adding up to 4-5 % of the viral dry mass. Glycolipids of rabies virus are identical with those found in the host cells (5).

The proteins of rabies virus comprise five structural proteins designated L, G , N, NS and M. The role and function of these structural virus proteins are (4,5):

1. Glycoprotein (G-protein)

Glycoprotein is the largest polypeptide with a molecular weight of 78,000-80,000 daltons. This glycoprotein comprises the basic units of the spikes and is probably the most important surface component of the virus. It is associated with the attachment of the virus to cell surface

and is also concerned with the virulence of rabies virus. Determinants associated with virulence have been located on G-protein using anti-G monoclonal antibodies. The G-protein is the specific antigen responsible for the production of neutralizing antibody and for conferring immunity to animal against a lethal challenge infection.

The rabies virus glycoprotein contains complement-fixing (CF) antigen. Immunolysis of infected cells in the presence of complement is the action of antibodies directed against the viral G-protein. Purified glycoprotein does not exhibit hemagglutination (HA) activity as does whole, intact virus in the presence of goose erythrocytes. The hemagglutin inresponsible appears to be associated with the integrity of the viral unit membrane.

The G-protein is a distinct antigen not cross-reacting with antibodies against N-protein. It can demonstrated by CF- and immunodiffusion tests. Immunofluores cent staining of infected cells by anti-G antibodies showed a characteristic cell membrane fluorescence devoid of intracytoplamic staining as with anti-N sera.

2. Ribonucleocapsid protein (N-protein)

The ribonucleoprotein is the second largest polypeptide of rabies virus linked to the viral RNA to form the helical ribonucleoprotein. It has a molecular weight of

58,000-62,000 daltons representing 31-34 % of the viral protein. Antibodies raised against N-protein have complement-fixing but no virus neutralizing activity. Withimmunofluorescent staining, stains intracytoplasmic inclusions (Negri bodies) (7).

N-protein preparations from rabies and rabies subgroups (Mokola and Lagos bat virus) are closely related by serological techniques, but virus neutralizing test using antivirion sera to rabies and rabies subgroups differs considerably (8). These findings suggest that N-protein represents the group-specific antigen of the rabies group of rhabdoviruses, whereas the G-protein apparently determines the serotype.

3. Membrane Proteins (M-proteins)

M1 and M2 are the membrane proteins with molecular weights of 35,000-40,000 and 22,000-25,000 daltons respectively. The larger membrane protein, M1, representing 8-10 % of the viral protein content is associated with the inner leaflet of the lipid bilayer of the viral envelope. M2 functions to connect the helical nucleocapsid to the viral envelope. It represents 10-13 % of the viral protein content. The antigenic and biological functions of these membrane proteins still remain vague.

4. L-protein and NS-protein

L-protein is a large protein with a molecular weight of 190,000 daltons. NS-protein of 47,000-55,000 daltons is a small protein, which locates together with the nucleocapsid. These two proteins are probably associated with the transcriptase activity necessary for the replication of rabies virus (4).

The structural model of rabies virus is outlined in Fig.2 (9):

1. The nucleocapsid is a single-stranded, right handed helix (6).

2. The surface projections are in a hexagonal arrangement (10,11) and are represented by the viral glycoprotein (12).

3. In the virion, the ratio of the 4 structural proteins G, N, M2 and M1 is approximately 1:1:1:0.5 (12,13).

The helical nucleocapsid containing RNA, N-, and NS-protein forms the central part of the bullet shaped virus partical. Nucleic acid strand is coverred with the N-protein within the nucleocapsid. How the NS-protein is embedded within this core is still unknown.

As with most enveloped viruses, infectivity of

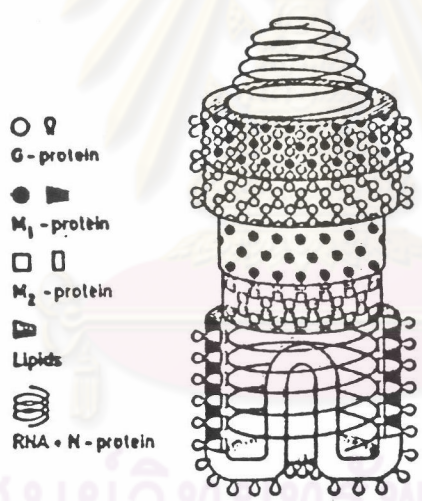

Figure 2. A structural model of rabies virus. By courtesy of Dr. Steven Vernon (Vernon et al., 1972).

rabies virus deteriorates rapidly at room or refrigerator temperatures in the absence of tissue proteins or added proteins (normal serum or albumin). Inactivation is much slower, however, in crude tissue extracts or in infected tissues stored in neutral glycerol. Infectivity is quite stable in frozen or lyophilized tissue extracts (14).

Transmission

Rabies infection is nearly always the result of a bite by a rabid animal (15,16). The saliva has been incriminated for the transmission of disease, because it contains the virus. The most important nonbite transmission route of rabies appears to be the respiratory (air-borne) route. Several reports have confirmed the fact that airborne transmission of bat rabies to human and animals is possible as experienced in Frio cave of Texas, in which free-tailed bats acted as source of infection to human (17). The transmission of rabies by ingestion (18) and transplacental transmission(19) have also been considered as possible. In several species of mammals, including dogs(20), cattles(19), bats(21) and laboratory rodents(22), rabies has been transmitted across the placenta from mother to fetus. This, however, does not seem to happen during human pregnancy (15).

Although rabies patients commonly carry rabies virus in their saliva, tear, and sputum during the first week of their illnesses, their has been no virologically

documented case of person-to-person transmission of rabies, except the corneal transplants (23,24,25).

Incubation period

In rabies the incubation peroid is more variable than in any other diseases, ranging from 4 days to (reportedly) many years. The short incubation period is usually associated with bites on the face, head and neck and is more common in children with severe or multiple bites as well as in experimental animals injected with large doses of virus.The average incubation period in facial bites is about 5 weeks, and about 8 weeks for bites on the limbs (15). In the 4 reported corneal transplant cases the incubation period was short, ranging from 22-33 days (15). Vibulbandhit kij reported that in 319 rabies patients admitted during 1971-1977 who had history of animal bite, the incubation period ranged from 4 days to 4 years (26). The four-day incubation period was the shortest ever reported after naturally occuring infection with street virus (26). In 1956, Wang reported a groups of 235 cases of rebies occuring in Taiwan from 1952-1955, seven years before rabies was eradicated from the island (27). In this series, the incubation period was less than 3 months in 85 % of cases, less than 1 year in 93 % and was between 21-90 days in 62 %. The correspondingfigures in the Thai series were 77,96 and 75 %, respectively.

Some of the very long incubation periods (19 years or more) which have been mentioned in the scientific literatures may probably be explained by a second more recent, but perhaps less dramatic, exposure, which the patient had forgotten. Another explanation could be that the virus lies dormant somewhere in the nervous system until it is reactivated by another infection or some other kinds of stress. Viruses of the herpes group behave in this way. The infection or stress that can reactivate the latent herpes viruses are some local mechanical irritants and fever. For example, herpes simplex type I virus may remain latent in the nervous system for months or years and can be reactivated by fever such as during a common cold or pneumonia. Human cases of rabies have been associated with colds and herpes simplex infections and in experimental animals, rabies encephalitis can be precipitated by strese or by injection of corticosteroids which are normally produced by the adrenal cortex at times of stress (28).

Pathogenesis

The pathogenesis of rabies begins when rabies virus enters a new host. The virus deposited in the tissue of the victim at the entry site is able to start its cycle of infection and multiplication.

The movement of rabies virus in nerves was demonstrated experimentally by amputation and nerve-section

experiments in animals infected with fixed virus. By electron micrograph, virus particles were demonstrated within the axon cylinders of nerves (29,30). The virus moves passively by the natural to-and-fro movement of the axoplasm (15). Inhibition of axoplasmic flow of the sciatic nerve by colchicine and vindblastine prevented the development of rabies in experimentally infected mice, just as nerve section did (31). No multiplication of the virus occurs in the axon cylinder, but replication occurs when the virus reaches the cell body of the neurone which contains the necessary apparatus for synthesis of RNA and protein and it is here that the amount of infectious virus in the animal is amplified.

Before the virus enters the nerves, it multiplies locally in muscle cells and is shed into extracellular spaces. Muscle spindles and motor endplates in striated muscles are implicated as the driving force for the virus to enter the nerve (29,30,32) Experiments by Watson et al indicated that the distribution of virus and virus antigen, one and six hours after inoculation, was closely related to acetycholine receptors (33). This suggests not only the involvement of motor end-plates, but also that the virus may utilize acetylcholine receptors by binding to them bofore entering the nerve. Tsiang, in his studies of neuronal function in rabies-infected bat brains, has confirmed the important role of acetylcholine receptors in the pathogenesis of rabies (34).

There are indications from the experiments of Murphy and his co-workers that antibody is effective in neutralizing the virus only during the period before it enters the peripheral nerves. Once the virus has entered the nerve axon it is carried centrally and amplication occurs at neuronal cell body. When it reaches the central nervous system the number of available neuronal cell bodies is enormously increased, leading to a similarly enormous increase in the amount of the virus in spinal cord and brain. The abnormal sensation, often felt at the site of the bite during the prodromal period, may be related to the entry of the virus into the dorsal root ganglia and its replication there (15).

In the early state of invasion of central nervous system the infection is relatively selective (35). The virus is initially localized in the neurones of the limbic system and as Johnson writes " this provides a fascinating clinicopathologic correlate (36). The alertness, loss of natural timidity,abnormal sexal behaviour,and aggressiveness that typify clinical rabies represent a diabolical adaptation of virus to selected neuronal populations--neurons capable of driving the host in a fury to transmit the virus to another host animal".

From the central nervous system, the virus spread centrifugally via efferent nerves of all types to the neural cells of virtually every organ and tissue in the body

--including, of course, the salivary glands. In these glands the virus undergoes further replication in acinar cells and the newly produced particles are budded directly into the salivary ducts, making the cycle of infection possible once again (15).

Rabies vaccine and Vaccination

Rabies is a hightly fatal infectious disease that affects all species of warm-blooded animals including human. Though this disease has been recognized since ancient time, the specific therapy still remains unknown. Therapeutic interventions therefore, rely on post-exposure prophylactic measures which include wound cleaning, rabies immunoglobulin and rabies vaccine. Nowhere is this principle better illustrated than in the treatment and prevention of rabies, where the time and site of probable infection are well defined, the incubation peroid is long and the progress of disease is characteristic and predictable. The long incubation period theoretically allows time for immune responses to vaccine to occur before the onset of the disease. In addition, passive administration of rabies immune globulin fill up the gap for the antiviral activity while waiting for the vaccine-induced immunity to occur.

Vaccine prepared from nervous tissue

1. Adult animal nervous tissue vaccines

Pasteur was the first to vaccinate a boy who had been bitten by a rabid dog with live attenuated adult rabbit spinal cord vaccine in 1885 (37). The vaccine was prepared by injecting a "fixed rabies virus" into a rabbit. When the rabbits showed signs of paralysis, their spinal cords were immediately removed and suspended in sterile jar over a dessicating agent at room temperature. The infected cord became almost non-virulent in about two weeks. This vaccine was called dried cord or Pasteur vaccine, which had been prepared by emulsifying small portions of the dessicated spinal cord in a salt solution. The treatment applied to the boy consisted 13 inoculations during the period of eleven days (38).

The year following the introduction of rabies vaccination,some 2500 people were treated. Failures and vaccine associated rabies deaths were reported due to the fact that very small amounts of living rabies virus survived in the vaccine after dissication (39,40). As a result, furtherimprovement of the vaccine was required. Fermi(41), Semple(42) and Hempt(39) modified the Pasteur's vaccine by using adult animal brain tissue for in vivo viral cultivation. Although the nervous tissue derived rabies vaccines had their origin in the Pasteur era,they are still being produced and used in several developing countries.

Semple, an English physician, working in India prepared rabies vaccine in which the virus in the inoculated

rabbit brain suspension was killed by incubating with carbolic acid (42). Semple vaccine was further modified by using sheep or goat brain to yield a larger volume. The bulk of the current world production of rabies vaccine is prepared by Semple's method and it was, and probably still is, the most widely used of all the rabies vaccines. For almost a century, the infected neural tissues of adult animals provided abundant sources of virus for the production of rabies vaccines and these notoriusly crude preparations have represented the major post-exposure preventive measure against fatal rabies.

The disadvantages of adult animal brain rabies vaccine were neurological complications and a high failure rate (43,44,45,46). Reactious usually occur after several doses of vaccine have been administered and range from mild tingling of the hands or feet to transient paralysis. Permanent damage to the nervous system sometimes occurs and even more rarely, reactions may be fatal. The incidence of neurological complications reported by Appelbaum and his coworkers varied between 1 per 600 to 1 per 1575 vaccinations (44). The frequency of neurological reaction in Thailand is estimated to be 1 in 2500 courses of these vaccinees and it has a mortality rate of 17 % (47). Studies have indicated that such neuroparalytic complicatious are due to the encephalitogenic property of the myelin proteins of the nervous tissue (48). Numerous workers have then tried to prepare safer vaccines by removing this unwanted component.

Rabies virus binds tightly to brain tissue, therefore these attempts have not been overwhelmingly successful (49).

2. Immature animal brain tissue vaccine

Myelin is absent from the nervous system of newborn animals such as the brains of suckling rabbits, rats and mice (50,51). This fact and the rapid multiplication of rabies virus to high titer in the brain of newborn animals, were exploited in the preparation of an ultraviolet-inactivated vaccine of high potency from suckling-mouse brain. The vaccine had successful preliminary trials (52,53). Such inactivaled suckling mouse brain vaccine has been used widely in rabies prophylaxis in the Latin American countries. However, the first 32 cases of neuroparalytic accidents with a rate of 1/7865 vaccinations and with a fatality rate of 21.9 % were reported from suckling mouse brain rabies vaccine (54).

Vaccines from non-neural tissues

1. Avian embryos

Vaccines prepared from virus grown in non-neural tissues have obvious advantages due to the absence of myclin. Adaptation of several strains of rabies virus to grow in avian embryos was first recorded 30 to 40 years ago (39, 55,56). Two vaccines containing live attenuated virus

grown in chick embryos have been widely and successfully used in animals but gave disappointing results when tested in man. During the same period an inactvated vaccine suitable for human use was developed from virus grown in duck embryos by Peck and his associates (57). In a clinical trial conducted by Greenberg and Childress, the results suggested that antibody was produced more rapidly in persons receiving this vaccine than in those receiving nervous tissue vaccine (58). Although this vaccine is free from the encephalitogen factors, its use is often accompanied by local reactions and some servious cases of anaphylaxis and neural involvement have been reported (59,60).

2. Tissue Culture Rabies Vaccines

2.1 The first generation of tissue culture rabies vaccine

Due to poor antigenicity, occurrence of impurities and high rate of treatment failure of the nervous tissue-derived rabies vaccines mentioned above (61,62,63,64, 65,66),continuous efforts were expended in the search for a highly immunogenic and safe antirabies vaccine which could be effectively used at low dosages for pre-and post-exposure treatments. Wiktor and his associates adapted a rabies virus strain to human fibroblast cell culture. Punification and inactivation of the virus produced in these cells led to a highly immunogenic vaccine, called "human diploid cell

rabies vaccine" (HDCV) , free of nerve tissue or other animal protein (67). Initial trials of HDCV in monkeys showed that a single dose gave much higher antibody response than did similar doses of brain tissue or duck-embryo vaccines, and that the animals were protected against subsequent experimental infection with wild strains of rabies virus. More importantly, it was demonstrated that a single dose given several hours after experimental infection protected more monkeys than did fourteen daily doses of duck-embryo vaccine (68,69). HDCV had extensive trials in many thousands of human volunteers in the United States, the Middle East, Europe and Asia including Thailand. The results of these trials were unanimous. All recorded a striking immune response and the absence of other than minor local reactions (70,71,72,73,74,75,76,77,).. After the administration of many millions of doses, the early promise of HDCV has been maintained. There have been only two recorded incidents of vaccine failure (78,79) and two cases of transient neurological disturbances (80,81). The two cases of vaccine failure most likely resulted from delayed administration of HDCV and the disregard for the use of rabies immune globulin (78,79).

Advantages of HDCV include its high efficacy, safety and only five to six doses are required for post-exposure treatment (VS 17-20 doses for Semple vaccine or suckling-mouse brain vaccine). Thus, human diploid cell rabies vaccine has become widely used in western countries, but the difficult production process and low yield of virus

make it too expensive for developing countries where the problems are great as in Thailand. There is a need to find an economical way of using the safe and effective vaccine.

The strategies for more economic use of vaccine are outlined :

a) develop new high potency vaccines costing only a fraction of the HDCV.

b) reduce schedules of immunization or give smaller quantities of viral antigen in each dose with an adjuvant.

c) inject vaccine with the alternative regimens such as intradermal (I.D.) route.

2.2 The second generation of tissue culture rabies vaccines

To find a cheaper but safe and effective vaccine, the searches for other suitable cell systems, vaccine strains and technologies have continued.Some of the cell types, hitherto considered unsuitable for human use, such as primary hamster kidney cells, have been reassessed and, provided that appropriate safety tests are applied, can be used as vaccine substrates. Furthermore purification techniques have advanced considerably during the last decade. For example, viral antigen can be physically separated from substrate and other impurities by sophisticated chromatographic or centrifugation methods. Such methods are applied in the production of safe and potent rabies

vaccines prepared in avain and several other cell systems (82). To date, several kinds of the second generation tissue culture vaccine which are safe, effective and cheaper than human diploid cell vaccine are introduced such as primary hamster kidney cell rabies vaccine (PHKCRV), primary factal bovine kidney cells rabies vaccine (FBKCV) and purified chick embryo cell culture vaccine (PCEC) (83,84,85).

A new tissue culture vaccine designated Purified Vero Rabies Vaccine (PVRV), a cheap, safe and effective vaccine has been developed. The fixed rabies virus, Pitman-Moore (PM) the same strain used in the HDCV production, has been adapted to grow in the Vero cell, a continuous, aneuploid cell line derived from Vervet monkey kidney cell. Vero cell can be adapted to the microcarrier system for higher cell population density and inturn a higher yield of rabies virus antigen has been achieved. Their cultivation in large fermenters makes large scale commercial vaccine production feasible, and such technique can reduce the cost of the vaccine production, Only 0.5 ml per dosage is required and 5-6 doses as HDCV are recommend for post-exposure treatment (86).

Alternative regimens for rabies vaccination.

Althought aforementioned new generation rabies vaccines are farless expensive than HDCV, they are nevertheless true that 6-dose regimen with newer tissue

culture vaccines such as those derived from Vero cells and primary chick embryo cells will still cost 5 to 10 times more than treatment with nervous tissue vaccine. To use potent tissue culture vaccines for more efficiently than at present, the alternative regimeus have been described above (in section 2.1). These regimens should meet the following desirable properties of a post-expsure treatment (87) :

1. The rapid induction of a solid immune response with few adverse reactions.

2. It should be as inexpensive as possible

3. Ideally, it should involve few clinic visits and as few injection sites as possible.

4. It should require little clinical skill i.e., it should be readily carried out by nurses and be practicable in the very young and elderly.

5. The preparation of vaccine from the storage stage should require little time and should be as straight forward as possible.

6. Finally, the immune response should not be overcome by passive immunization or by commonly prescribed drugs ; it must be demonstrable in the under nourished and in persons with parasitic infestation.

A. Incorporating adjuvant into the vaccine

There have been several studies of the antigenicity of low concentration HDCV mixed with adjuvant. In the first

study, Kuwert et al. (88) compared day 28, day 56, and day 70 antibody responses to either 1.0 ml dose of standard HDCV or 10-fold diluted vaccine in 0.1 % $Al(OH)_3$. Both vaccines were given in 1.0 ml volumes on days 0, 28 and 56, Both groups attained almost identical titers after several months, but the early antibody response was not investigated. This study had similar findings to another study in which the vaccine diluted in adjuvant was given on day 0,3,7,14 and 28 (63).

In summary, the adjuvant effect of 0.1 % $Al(OH)_3$ can almost completely compensate for a 90 % reduction in antigen content with regard to the overall height of the antibody response, but the appearance of antibody during the early critical period after vaccination seems much delay.

B. Intradermal vaccination

A great many studies attest to the safety and immunogenicity of alternative regimens of HDCV. Several studies showed that multisite intradermal immunization resulted in higher seroconversion rate by day 7 and comparable peak antibody response whereas only one quarter of the standard intramuscular HDCV dose was used (63,64, 89). Besides the more rapid antibody response,the intradermal regimen was more effective than the intramuscular regimen in inducing prompt cell-mediated immune response to the virus as assayed by the antigen-stimulated lymphocyte transforma-

tion test (90). The advantages of the intradermal regimens are cost saving of 75 percents, high antibody and rapid cell-mediated immune responses as compared to other regimens. Protective efficacy of the intradermalregimens has been demonstrated in both animals and man (63,64,89,91). The side-effects of intradermal regimen are mild, mainly local and self-remitting (89).

C. The 2-1-1 regimen

There has been a new modified intramuscular regimen for postexposure use designated "2-1-1". This regimen cansists of 2 separate doses given on day 0, followed by one dose each on day 7 and 21. It has advantages over the standard regimen (1 dose of vaccine on day 0,3,7,14,28 and 90) in terms of immunogenicity and economy (92). The 2-1-1 regimen saves 2 dosesof vaccine and 3 clinic visits.

As reported previously by Kuwert et al, the omission of the day 3 dose from the standard regimen dose not affect antibody response to HDCV (93). Doubling the antigen load on day 0 provides a powerful initial immunological lift-off, and boosting on day 7 makes vaccination on day 3 and 14 redundant (92). One-site vaccination with a large dose of vaccine bears the concern of possible hindrances or mishaps that might impair the efficacy of the initial immunization. This danger can be avoided by bllateral or multi-site

vaccination on day o.

Vodopija and his associates performed clinical studies with three different tissue culture rabies vaccines; HDCV, FBKC and PCEC using the modified 2-1-1 regimen. HDCV was administered intramuscularly 0.5 ml(half dose)x4 on day 0 into both deltoid and gluteal areas, followed by 1.0 ml dose on day 5 vs.day 7 and again on day 30. By day 14 antibody titers were >0.5 iu/ml (93% of the vaccinees). All subjects seroconverted by day 30 .The second vaccination on day 7 gave higher antibody response than when given on day 5. Antibody titers on day 90 were above 0.5 iu/ml (94). The two studies with FBKC and PCEC gave similar results to the HDCV study (95,96).

Results from all of the studies emphasized the importance of the timing of the second vaccination. Day 7 seemed to be optimal timing,well attuned to the biological pace of specific immunity maturation (93,94,95). Upon achieving immediate protection against the threat of rabies virus replications every additional injection of the vaccine technically represents a booster. The 4 th vaccine dose (3rd clinic visit) should stimulate the inborn slower maturation of the specific immune response. The 3 rd visit was on days 28/30 from all of the studies described above.

Vaccines and Immunity

The body has more than one way of expressing

specific protective immunity to infection and in responding to a vaccination. One of these is the production of soluble substances associated with the serum protein of the blood. These are usually described as "circulating" or "humoral" antibodies. The other response is associated directly with cellular activity and is known as "cell-mediated" immunity. The respective roles of these two aspects of the immune response to rabies are still under investigation.

Antibody responses

Antibody has remained a central theme in the development of immune response to most vaccines. The resistance induced by rabies vaccine is always associated with antibody production. The relative ease with which antibody can be measured has made it a convenient, though not necessarily accurate, guide to the efficiency of vaccines.

Rabies vaccination does not induce any measurable antibody in brain or cerebrospinal fluid in man and its utility in post-exposure therapy is limited to the brief period before virus attaches to and becomes inaccessible in target cells. Nevertheless, neutralizing antibody, actively induced by vaccine or transferred passively early in rabies infection, has long been regarded as a key factor in the post-exposure protection of man. The most convincing evidence of the role of antibody in man was demonstrated.

This unique and dramatic incident occurred in Iran where a rabid wolf bit 29 people in succession (97). Seventeen also received one or two injections of anti-rabies serum ; only one of these died, compared with three of the remaining twelve who received only vaccine. The figure was even more dramatic if only those with severe head wounds were considered : 75 % of those who had only vaccine died compared with 14 % of those receiving serum and vaccine. Vaccination is therefore unlikely to be effective in severe infections where the incubation period may be short. Many individuals dying of rabies have very high levels of neutralizing antibody late in their infection, suggesting that antibody appearing at this time does not affect the lethal outcome of the disease (82).

Some workers believe that antibody alone may only prolong the time to death and that some antibody components are actually involved in the disease process or pathogenesis of rabies (98). Some evidence implicates antibody as a component in the immunopathogenesis of rabies, either being involved in immune cytolysis (99), or in the enhancement of infectivity when present in low concentration (68,100,101).

There seems to be no doubt that antibody is involved in the response to infection and that it may have adual role, either protecting against rabies or, accelerating death from the disease. Many animal experiments have demonstrated that antibody protects if it is administered

before virus has reached the central nervous system. The administration of antirabies serum as well as vaccine, within 24-48 hrs of infection, is recommended practice in all severe exposures to rabies (68).

Cell-mediated immunity

Attempts to assess the relative importance of cellular response to rabies vaccine owe more to observations in mice than to very many in man. Both depletion of circulating lymphocytes with anti-lymphocyte serum (102) and transfer of resistance with immune spleen cells are unsuccessful in establishing of relative role of T cells (103). The studies with nude athymic mice show that thymus is necessary for protection (104,105,106), but that antibody production is probably T cell-dependent (103). None of these early results permit any conclusion other than T cells are essential for the immune response to rabies vaccine.

Several attempts to dissect the humoral from the cellular response to vaccine were made in mice treated with cyclophosphamide. Measurements of delayed-type hypersensitivity and of the development of lymphocyte cytolytic for rabies infected target cells were made in mice vaccinated and treated with cyclophesphamide. The results provided strong arguments for the participation of cellular immunological mechanism (107,108,109,110,111). Wiktor and his co-workers demonstrated a strong, specific, cell-

mediated cytotoxic response to rabies vaccine in mice. Optimal cytotoxicity was demonstrated when the effector cells and the virus-infected target cells shared the same major histocompatibility (MHC) antigens. However, there is no direct evidence to show that such a cellular response is protective (112). The results are mouse strain-dependent, and the experimental conditions are far from the usual circumstances in which humans become infected and vaccinated (113).

Although less frequently reported, specific lymphocyte transformation, another in vitro correlate of specific T cell response, occurs in most but not all human vaccinees given cell-culture rabies vaccines (114,115,116). Non-immeme, human lymphoid cells and human rabies antibody are also able to lyse rabies-infected target cells. This antibody-dependent cellular cytotoxicity (ADCC) has been observed in vaccinated subjects (117). Little atlention has been directed toward the participation of macrophages in the immune response to rabies vaccine. They are known to be involved in the regulation of in vitro antibody responses and in non-specific resistance (118,119).

Current immunological evidences suggest that in general, T lymphocytes are concerned with monitoring other cells with virus-associated changes on their surfaces. Cytolytic T cell subsets (Tc) react with and destroy these virus-infected targets, provided they corry the same

MHC antigens. Furthermore, this cell-mediated system is believed to interact solely with virus-infected cells but not with the free virus (120). Whether these cellular effects are protective or whether they contribute to pathogenesis needs clarification.

Immunologic responses to Purified Vero Cell Rabies Vaccine

Svjetličić and his coworkers reported studies with the PVRV in 100 healthy volunteers in Yugoslavia. The vaccine was well tolerated and in general it evoked titres comparable to those of HDCV (121). The neutralizing antibody appeared on day 14 and the titers were obove 0.5 iu/ml throughout 100 days (121). Chadli studied the post-exposure use of PVRV in Tunisia in 75 subjects bitten by rabies-suspect animals. Four to six subcutaneous injections were given on day 0,3,7,14,30 and 90 ; 0,3,7,14 and 30 ;and 0,3,7 and 14. Seroconversion ranged from 10.5 to 26.5 % on day 7 and were 100 % on day 14. All of these people were in good health one year after commencement of treatment (122).

Phanuphak and his associates (123) compared the administration of full course intramuscular injection of PVRV on day 0,3,7,14 and 28 with 4-site intradermal injection of 0.1 ml each on day 0,3 and 7 followed by a single ID injection on day 28. Both reginuns resulted in 100 % seroconversion by day 14. The 4-site ID group had a significantly higher neutralizing antibody titer than the I.M. group at all time periods. The cell-mediated immune response

induced by 4 site I.D. regimen occurred one week sooner than the full dose I.M. These results were similar to the immunogericity of I.D. (89,90).

Suntharasamai et al (124) studied the protective efficacy of PVRV in patients bitten by animals with proven rabies. PVRV was given intramuscularly y on day 0,3,7,14,28 and 91 with simultaneous administration of 20 iu/kg of human rabies immune globulin (HRIG) to ones with severe exposure. Rabies neutralizing antibody was demonstrated on day 14 and persisted for 1 year in every case. There was no significant suppression of the antibody response by HRIG. All patients are alive and well after 1 year.

Of special interest is the elaboration of the 2-1-1 regimen in conjunction with the Purified Vero Cell Rabies Vaccine (PVRV). The 2-1-1 regimen reduces the number of clinic visit from 6 times to 3 times and saves 2 doses of vaccine.

This report compares the immunogenicity of the 2-1-1 regimen of Purified Vero Cell Rabies Vaccine with the standard full-dose intramuscular regimen, both for the humoral and cell-mediated immune responses. The suppressive effect of the concurrently administered human rabies immunoglobulin (HRIG) and equine rabies immune globulin (ERIG) with the 2-1-1 regimen was also studied

Objectives

1. Study the humoral and cell-mediated immune responses to intramuscular 2-1-1 regimen of Purified Vero Cell Rabies Vaccine (PVRV) as compared to the standard full-dose intramuscular regimen

2. Assess the suppressive effects of the administered human rabies immune globulin (HRIG) and equine rabies immune globulin (ERIG) with the 2-1-1 regimen

3. Compare the antirabies antibody titers estimated by Indirect Enzyme Linked Immunosorbent Assay (ELISA) with the neutralizing antibody measured by Rapid Immunofluorescent Focus Inhibition Test (RIFFIT).