CHAPTER I

INTRODUCTION

Rabies is primarily a canine disease. A close relationship is observed between the number of rabid dogs and incidence of human rabies. In Thailand, dog-bites are responsible for 97% of human rabies deaths (Thongcharoen *et al.*, 1983). Canine rabies predominates in developing countries which include Asia, Central and South America,and Africa. World Health Organization reports 50,000 annual rabies death worldwide. Rabies death annually reaches 25,000 in India, 200-300 in Thailand and an estimated 5,000-7,000 in China (Wilde *et al.*, 1991; Baer, 1988). In the United States, fatality among humans exposed to rabies is relatively low. Nevertheless, about 30,000 people require postexposure rabies immuno-prophylaxis, which costs $100 million annually. Wildlife is the main source of the residual rabies threat in the developed world. These wild animals include skunks, raccoons, foxes and bats. In North America and Europe, several terrestrial species are involved in the cycle of wildlife rabies. The fox is the largest rabies reservoir and has been chosen as the target animal for the oral immunization efforts. Vampire bat rabies and the resultant bovine rabies are important components of the wildlife rabies, where they are enzootic from northern Mexico to northern Argentina.

The goal of rabies control in domestic and wild animals is the reduction or elimination of human rabies. Many advances have been made for the provision of better treatment of victims bitten by rabid animals. These advances include local wound treatment, the use of antiserum for people bitten by known or suspected rabid animals, and the production of the markedly more effective and safer cell culture derived vaccine.

Once infection occurs, the clinical course can be divided into 5 stages (Hattwick and Greff, 1975):

1. Incubation period of variable length.
2. Prodome with headache, fever, nausea, vomiting and local paresthesia at the site of the bite.
3. Acute neurogical phase characterized by paresis or paralysis, hydrophobia, aerophobia, confusion, hullucination, and hyperactivity.
4. Coma
5. Death or recovery.

One of the most puzzling aspects in human rabies is that it may present in one of the two forms, encephalytis or paralytic, which are analogous to canine 'furious' and 'dumb' rabies. The paralytic manifestation is seen in at least one-thirds of the cases. Patients with encephalitis usually have a rapid and progressive course. Survival time after the onset of symtoms to death is shorter in encephalitis. The pathogenetic mechanisms that determine encephalitis and paralytic rabies are still unclear. Determinant may involve one or a cooperation of these factors: certain animal vectors, different virus strains, location of the wound and host immune response.

Although diagnosis of rabies virus has improved, it still needs laboratory facilities and equipments which are not always available. Direct detection of viral nucleocapsid inclusions on brain smears with a fluorescent antibody test (FAT) has become a basic method for routine rabies diagnosis (Dean and Abelseth, 1973). Viral isolation by newborne mice intracerebral inoculation test (MIT) needs at least 2-3 weeks before the rabies symptoms develop (Koprowski, 1973). Rabies tissue culture infection test (RTCIT) has reduced viral isolation to only one day (Rudd *et al.*, 1980). Detection of viral genetic material by PCR technique has become possible. This technique has proven to correlate well with FAT and MIT (Sacramento *et al.*, 1991). All these rapid diagnosis techniques are available only in laboratories with UV microscopes, cell culture facilities and DNA thermal cyclers. Recent advances in recombinant DNA technology have resulted in the access to abundant supplies of cloned DNA fragments. Nucleic acid hybridization techniques have recently been utilized in rapid diagnosis of a variety of infectious diseases (Lowe, 1986). Mass screening of biological specimen could be carried out rapidly and conveniently without the use of sophisticating equipments. This approach was applied in this study to the detection of rabies virus RNA in brain specimen. Dot hybridization could be further applied to analyzed rabies RNA species within brain.

Little was known about the genetics of rabies virus since Louis Pastuer successfully isolated this virus from rabid canine and led the way in fighting against rabies with his immunological treatment a century ago. The first rabies gene sequence was reported in 1981 (Anillionis *et al.*, 1981), and a molecular basis for the definitive classification of rabies virus by antigenic analysis was subsequently established. Since then, the knowledge of rabies virus molecular structure has enabled one to resolve the genomic organization of rabies virus, to determine the primary nucleic acid structure of entire rabies virus genome and the amino acid sequence of the rabies

virus structure and to correlate viral structure with known biological and immunological functions.

Rabies virus is a member of rhabdoviruses possesses a non-segmented, negative-stranded RNA genome. Complete nucleotide sequencing of rabies virus genome reveals that its genome organization is similar to that of vesicular stomatitis virus (VSV), the prototype of rhabdoviruses. The 11.9-kb genomic RNA contains 3' leader sequence, five open reading frames, coding for nucleoprotein (N), non-structural protein (NS), matrix protein (M), glycoprotein (G) and RNA polymerase (L), intergenic sequences and 5' noncoding sequence. Two distinct types of events in RNA synthesis are directed by this structure: transcription and replication. Transcription of the genome leads to the synthesis of a short leader RNA followed by the synthesis of five mRNAs, whereas replication leads to the synthesis of full-length copies of viral genome. The knowledge of transcription and replication of rabies virus has not been clarified. It has been supposed that the transcription and replication mechanisms of rabies virus are resemble to those of VSV. However, details of their RNA synthesis may be different and several features which are not shared in common by both viruses are known. For instance, only rabies virus, not VSV: 1) requires as much longer period to replicate in the cell; 2) has a gene-like region between the G and L genes in the genome which might regulate expression of the L gene (Tordo *et al.*, 1988); 3) has phosphorylated nucleoproteins (Sokol and Clark, 1973; Sokol *et al.*, 1974); and 4) constantly forms cytoplasmic inclusions. Molecular events in rabies virus-infected cells have not been so extensively studied as VSV, particularly, the transcription and replication which still remain obscure. Characterization of rabies virus transcription may significantly contribute to the understanding of the distinguished mode of viral multiplication from the VSV. In addition, if transcription of one viral gene is linked to that of another, then specific inhibition may lead to the disruption of the life cycle of that particular virus. With the advent of gene therapy by specific agents such as synthetic oligonucleotides or anti-sense RNA, it is possible to impair and prevent expression of a given gene. We hope that the knowledge of transcriptional events may provide the significant information leading to intervention with the life cycle of virus and thus perhaps complement other attempts at antiviral therapy.

The Polymerase Chain Reaction (PCR) is an *in vitro* method for the enzymatic synthesis of specific DNA sequences, using a pair of oligonucleotide primers that hybridize to opposite strand and flank the region of interest in the target DNA. A repetitive series of cycles involving

template denaturation, primer annealing, and the extension of the annealed primers by DNA polymerase results in the exponential accumulation of a specific fragment whose termini are defined by the 5' ends of the primers. Since the products synthesized in one cycle can serve as a template in the next, the number of target DNA copies approximately doubles at every cycle. Thus, 20 cycles of PCR yields about a million-fold (2^{20}) amplification. Reverse transcription coupled to the PCR, so-called RT-PCR, has been proven a powerful technique for analysis of RNA transcripts especially in low copy number (O'Brien *et al.*, 1993). Once the first cDNA strand is synthesized, *Taq* polymerase can be added to promote the synthesis of the second strand. Since the sensitivity and specificity of the RT-PCR has been addressed in detection of RNA transcript as little as picogram amounts, and the detection of newly synthesized transcripts requires a high sensitive and reliable technique, application of RT-PCR for monitoring the transcriptional events of rabies virus will be carried out in this study.

The purpose of this study was two fold:

1. To develop suitable probes which would allow for specific screening of individual rabies viral RNA species. Such probes would make it possible to screen a large number of brain samples without the use of radioactive agent.

2. To determine the transcriptional events of rabies virus genes in BHK-21 cells. The primary rabies RNA transcripts was analyzed by RT-PCR technique. The result is usuful to the understanding of the *in vivo* transcription of rabies virus.

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