# CHAPTER IV

# Results

## 1. Regional distribution of rabies virus, cytochrome C antigen and apoptosis in CNS.

Rabies virus, cytochrome C antigen and apoptosis were detected in most but not all examined regions from patients with rabies (Table 3 and 4).

### 1.1 Rabies viral antigen

The overall regional distribution of rabies viral antigen was roughly similar in terms of number and location to that previous report (ref.). Antigen-containing neurons (figure 5) were found predominantly in the brain stem and spinal cord, dorsal and ventral horn neurons, thalamus and basal ganglia particularly in patients who had survival periods of 7 days or less regardless of clinical types (H1, 5, 6 in furious and H4 in paralytic group). Those who died later than 7 days had rabies viral antigen disseminated throughout the whole neuraxis.

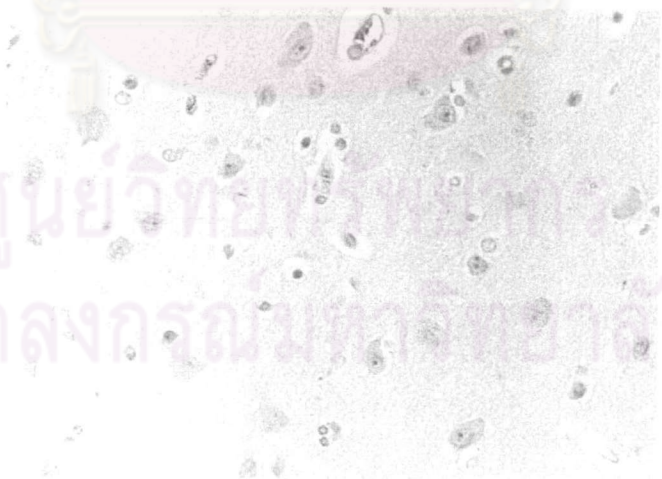

**Figure 4 :** Negative control staining in rabies infected tissue 200X

(result was similar in non-rabies infected tissue-not shown here)

**Figure 5 :** Rabies virus staining 200X

### 1.2 Apoptosis detection

#### 1.2.1 Cytochrome C antigen

Evidence of mitochondrial outer membrane permeabilization in this study was detected by demonstration of cytochrome C antigen. In furious group with a shorter survival period of 7 days or less (H1, 5 and 6), there was a discrepant result between the degree of rabies positive and cytochrome C positive neurons in spinal cord. This was also noted in rabies positive brainstem regions (midbrain, pons and medulla) of Patient H1 who had no evidence of MOMP in such region. This patient died within 5 days after onset. In patient H3 who had a survival period of 8 days, similar degree of rabies and cytochrome C antigen could be demonstrable in thalamus, basal ganglia and brainstem but yet lesser degree of cytochrome C leakage was still observed in thoracic cord. Such discrepancy was observed in paralytic Patient H4 (survival period of 7 days versus 16 and 13 days in H2 and 7 in the same paralytic group). There was no cytochrome C detected in thalamus and only minimal degree (of 1+) could be demonstrated in cervical and thoracic cords where 3+ antigen positive neurons were found. Brainstem is vital

brain structure in maintaining consciousness and alertness and is part of reticular activating system.

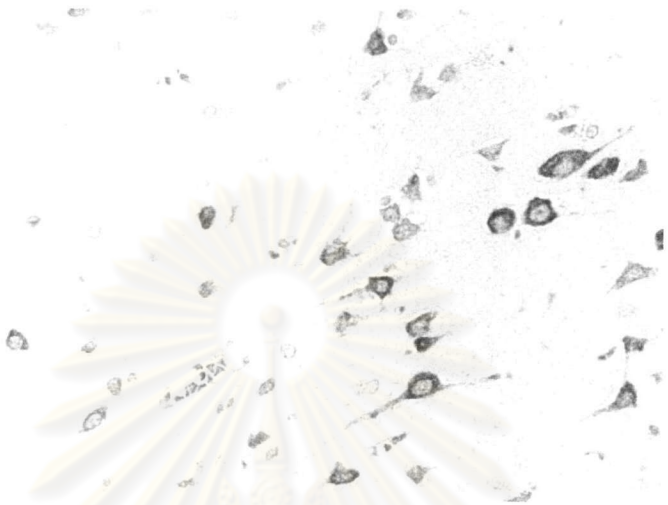

**Figure 6 :** Cytochrome C staining 200X

### 1.2.2 TUNEL assay

Apoptotic cells as demonstrated by TUNEL assays were found throughout the whole neuraxis. There was no significant correlation between short or long survival period, amount of rabies antigen positive neurons and degree of apoptosis in various CNS regions.

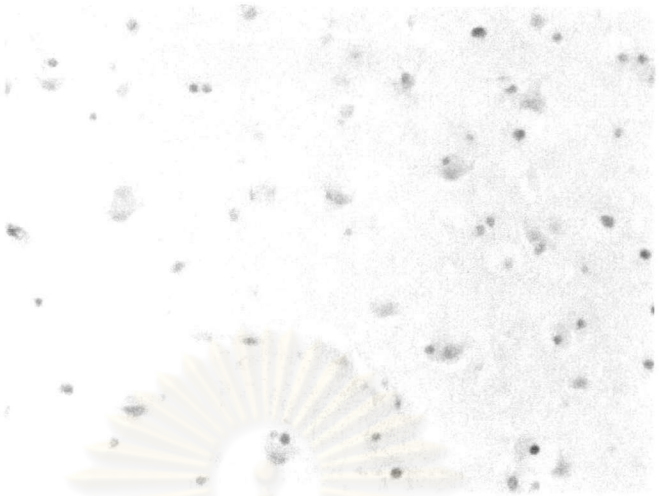

**Figure 7 :** TUNEL staining 200X (neuronal-negative)

**Figure 8 :** TUNEL staining 200X (neuronal-positive)

**Table 3 :** Distribution of rabies virus, cytochrome C and TUNEL assay in CNS of human encephalitic rabies.

| Patient No. | Survival period | Antigen | Frontal | Temporal | Hippocampus | Parietal | Occipital | Thalamus | Basal ganglia | Cerebellum | Midbrain | Pons | Medulla | Cervical | Thorasic | Lumbar | Sacrum |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Encephalitis** | | | | | | | | | | | | | | | | | |
| H1 | 5 | Rabies | 0 | 0 | 0 | 0 | 0 | 3 | 2 | 1 | 3 | 3 | 3 | 2 | 1 | 3 | N/A |
| R hand L leg | | | | | | | | | | | | | | | | | |
| L breast | | Cyto C | 0 | 0 | 1 | 0 | 0 | 3 | 1 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | N/A |
| H3 | 8 | Rabies | 2 | 3 | 3 | 4 | 3 | 3 | 4 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| R ankle | | Cyto C | 0 | 3 | 4 | 0 | 2 | 4 | 4 | 1 | 4 | 4 | 4 | 3 | 1 | 3 | 3 |
| H5 | 4 | Rabies | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 4 | 4 | 4 | 3 | 3 | 4 | 4 |
| buttock | | Cyto C | 2 | 1 | 4 | 3 | 4 | 4 | 4 | 2 | 4 | 4 | 4 | 0 | 0 | 2 | 2 |
| H6 | 5 | Rabies | 2 | 0 | 1 | 3 | 0 | 2 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| R buttock | | Cyto C | 1 | 3 | 2 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 3 | 0 | 0 | 0 |

**Table 4 :** Distribution of rabies virus, cytochrome C and TUNEL assay in CNS of human paralytic rabies.

| Patient No. | Survival period | Antigen | Frontal | Temporal | Hippocampus | Parietal | Occipital | Thalamus | Basal ganglia | Cerebellum | Midbrain | Pons | Medulla | Cervical | Thorasic | Lumbar | Sacrum |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Paralysis** | | | | | | | | | | | | | | | | | |
| H2 | 16 | Rabies | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | N/A |
| finger | | Cyto C | 0 | 0 | 0 | 0 | 1 | 3 | 3 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | N/A |
| H4 | 7 | Rabies | 2 | 1 | 2 | 2 | 2 | 3 | 2 | 2 | 4 | 4 | 4 | 3 | 3 | N/A | N/A |
| L calf | | Cyto C | 2 | 0 | 2 | 2 | 3 | 0 | 3 | 2 | 4 | 4 | 3 | 1 | 1 | N/A | N/A |
| H7 | 13 | Rabies | 2 | 3 | 2 | 3 | 3 | 4 | N/A | 3 | 4 | 4 | 4 | 2 | 3 | N/A | 4 |
| R leg | | Cyto C | 2 | 3 | N/A | 4 | 3 | 3 | N/A | 1 | 4 | 2 | 4 | 0 | 1 | N/A | 1 |

## 2. Morphology of white matter structures.

### 2.1 Tubulin and myelin basic protein (MBP) antigen

Tubulin and MBP antigen patterns in the CNS of both encephalitic and paralytic rabies were similar and unremarkable.

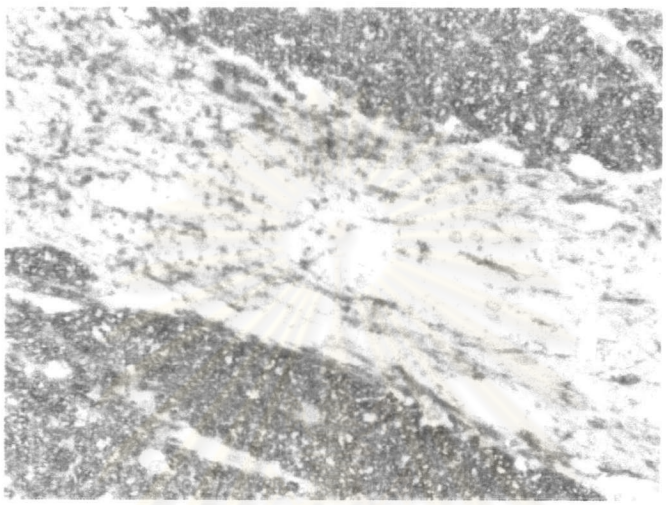

**Figure 9:** MBP staining 200X (white matter)

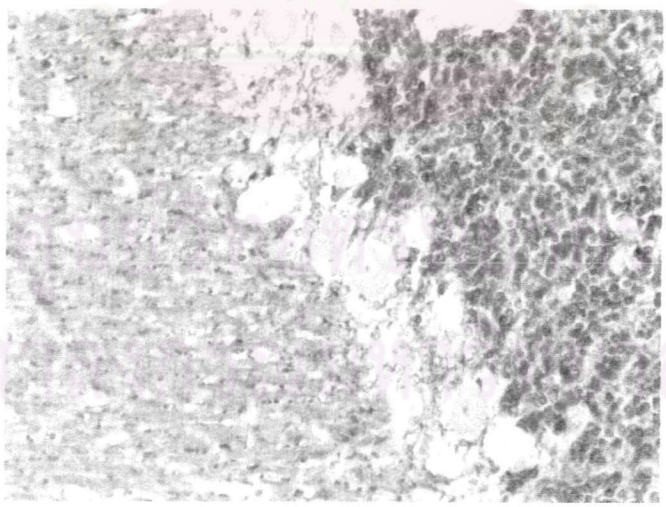

**Figure 10 :** MBP staining 200X (grey matter)