

# ISOLATION OF INFLUENZA A VIRUS IN REYE'S SYNDROME

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## INTRODUCTION

Reye's syndrome, or encephalopathy with fatty degeneration of the viscera, is an acute and often fatal childhood illness in which signs of brain swelling and liver enlargement develop rapidly (Olson *et al.*, 1970). The course of the disease is characteristic, and, once unconsciousness has developed, it can be diagnosed without difficulty. This is a common disorder in north-eastern Thailand and probably affects hundreds of children annually (Olson *et al.*, 1970; 1971). Sporadic cases are also found in the other parts of the country (Visudhiphan, unpublished data).

The definite aetiology and pathogenesis of Reye's syndrome remain undetermined; toxin, and infectious processes have been implicated (Becroft, 1966; Glasgow and Ferris, 1968; Glick *et al.*, 1970; Reynolds *et al.*, 1972). Olson *et al.*, (1970) believed that the epidemic cases reported in north-eastern Thailand were caused by aflatoxin which is produced by *Aspergillus flavus*. Substantial epidemiologic evidence for an aetiological association between Reye's syndrome and virus infection has been recorded especially in Europe and United States (Reynolds *et al.*, 1972; Cullity and Kakulas, 1970; Linnemann *et al.*, 1974). However, there are no case reports of virus isolation or any evidence of virus isolation or any evidence of virus infection by demonstration of the virus antibody in Southeast Asia.

Two consecutive cases of Reye's syndrome were studied and reported herein. Both patients, who lived in the Bangkok area, survived their illness after exchange blood

transfusion. Definite evidence of virus infection was demonstrated by four-folds increases of hemagglutination inhibition titers for influenza A virus in both patients. Influenza A virus of the same typing was also isolated from the nasopharynx of case 1. This is the first evidence that Influenza virus A is most likely to be the cause of Reye's syndrome in Thailand.

## CASE REPORT

**Case 1:** A 16-month-old girl was admitted to the Ramathibodi Hospital, Bangkok in July 1974 with a history of respiratory tract infection for three days. On the night before admission she had been unconscious, hyperventilation and had two generalized convulsions. Past history did not disclose any important exposure to toxin. She was well nourished, comatose and had deep strenuous respiration, rate 60/min. The rectal temperature was 39°C, pulse 110/min, and blood pressure 110/80 mm Hg. The general examination revealed mild dehydration, Heart and lungs were normal. The liver was 2 cm enlarged. The pupils were dilated but reacted slowly to light. The fundi were normal. There was generalized hypotonia and diminished tendon reflexes, but a positive Babinski's sign was elicited bilaterally.

A lumbar puncture was performed; pressure was 350 mm H<sub>2</sub>O; sugar, 36 mg%; protein, 27 mg%; and no white blood cells were seen. Liver-function tests at the time of admission were normal with the exception of a serum-glutamic-oxaloacetic-transaminase

(SGOT) level of 560 Sigma units, and a serum-glutamic-pyruvate-transaminase (SGPT) of 1,020 units. The blood sugar was 84 mg% (during intravenous infusion with 50% glucose). Blood salicylate level was 2 mg%.

Exchange blood transfusions (two volumes each) were performed about two and twelve hours, respectively, after admission, together with intravenous Dexamethasone and 50% glucose in water. The patient's condition gradually improved in a few days and the transaminase levels returned to normal within a week. The liver biopsy, which was performed on the third day of admission showed severe fatty change in the parenchymal cells, and no glycogen was detected in alcohol-fixed tissue.

**Case 2:** A 18-month-old boy, admitted to the Ramathibodi Hospital a few weeks after the first case, have had an upper respiratory tract infection for a week, had been feverish and vomiting for two days. On the morning before admission he lost consciousness and was breathing rapidly. Examination on admission revealed a comatose patient and mildly dehydrated, with marked hyperventilation. The rectal temperature was 39.2°C. The liver was 3 cm enlarged. The pupils were dilated but reacted to light. There was generalized hypertonia and bilateral unsustained ankle clonus. Deep tendon reflexes were hyperactive and with bilateral positive Babinski's sign. Occasional decerebrate posture was also observed during deep pain stimulation.

The blood sugar was 16 mg%. Blood salicylate level was 4 mg%. Liver function tests at the time of admission revealed no significant finding except SGOT level of 318 Sigma units, and SGPT level of 252 Sigma units. A lumbar puncture was performed; pressure was 300 mm H<sub>2</sub>O, sugar 20 mg%, protein 34 mg% and white blood cells were negative.

Two exchange blood transfusions, two volumes each, were performed about three and fifteen hours, respectively, after admission. Intravenous Dexamethasone and 50% glucose in water were also given. The patient's neurological symptoms and general condition gradually improved on the following day and returned to normal within a week. Liver biopsy was performed and showed severe fatty changes in the parenchymal cells, similar as in case 1.

**Virology:** Nasopharyngeal and throat swabs were obtained from patients for viral cultures. Specimens were collected in transport medium (medium 199 with antibiotics). These were kept at 4°C before transport to the virology laboratory. 0.2 ml of each specimen was inoculated into each of three types of cell culture, including primary rhesus monkey kidney, human fibroblastic lung (W 1-38) and K.B. cells. All cultures were incubated at 36°C and examined daily for evidence of cytopathic effects. All negative cultures were passed a second time in tissue culture. 0.2 ml of each specimen was also inoculated into the amniotic sac of a 9-10 day old chick embryo. All cultures were incubated at 35°C for 72 hours and it was found that the amniotic fluid harvested showed hemagglutinating activity with chick red blood cell. Influenza virus was identified by the hemagglutination inhibition test using strain specific antisera of Influenza A/Hong Kong/8/68, A/England/42/72 and A/Port Chalmers/1/73. Serum from acutely ill and convalescent patient was frozen at -20°C until test for viral antibodies. Hemagglutination inhibition antibody tested was according to the method of Robinson and Dowdle (1969).

Influenza A/England/42/72 strain was cultured from nasopharyngeal swab from the first case, but not from the second case.

Both patients had a four-fold increase in hemagglutination inhibition antibody to influenza A/England/42/72 (Table 1).

Table 1  
Virus isolation and serologic studies.

Case	Virus Isolation Influenza A/Eng./ 42/72	Hemagglutination Inhibition, serum titers, Influenza A/Eng./ 42/72
Case I		
Admission	+	1 : 10
13th day	-	1 : 40
32nd day	-	1 : 64
Case II		
Admission	-	1 : 32
19th day	-	1 : 128

## DISCUSSION

The syndrome of encephalopathy and fatty degeneration of the viscera was first described in detail by Reye in 1963. The clinical symptomatology of this illness is well documented (Reye *et al.*, 1963; Olson *et al.*, 1971). The syndrome has been reported worldwide from Australia (Reye *et al.*, 1963), Thailand (Olson *et al.*, 1970; 1971), Canada (Norman *et al.*, 1968) and the United States (Linnemann *et al.*, 1974). Of about 500 cases which have been documented, the majority had a fatal outcome. There are only a few reports which show a significant decrease in morbidity and mortality after peritoneal dialysis or exchange blood transfusion (Huttenlocher, 1972; Samaha *et al.*, 1974). The two patients were admitted in a moribund state, treatment in both cases was by exchange blood transfusion. The first case recovered with mild residual neurological deficit, especially the motor function. However, the second patient had complete recovery without any residual effect. This finding confirmed the previous report that early exchange blood transfusion would yield a very good outcome (Huttenlocher, 1972).

The clinical history in most of the patients with Reye's syndrome is suggestive of antecedent viral illness. It has been reported that cases occurring in clusters are most commonly associated with an increased incidence of influenza B in the community (Reynolds *et al.*, 1972). Virus isolation has been successful in only a dozen of the reported patients with Reye's syndrome. Most of them were from postmortem specimens, and there was no definite confirmation of the infection by serology. Linnemann *et al.*, (1974) reported twenty-four cases of Reye's syndrome from Cincinnati, Ohio. The epidemic coincided with an epidemic of influenza B, intermediate type. In 18 of 23 cases (78%) in which viral studies were done, an acute viral infection was found in either the patients or a close contact with similar prodromal symptoms. Influenza B infection was confirmed in 12 patients and 12 contacts, although influenza A, parainfluenza, adenovirus, and varicella-zoster virus infections also occurred.

In the first case of this report we could isolate the influenza A virus from the nasopharynx and confirmed that this virus was most likely to be cause of illness by demonstration of four-fold increases in the titer in the serum of the same patient. In the second case the evidence of recent virus infection was also clearly demonstrated by rising serum titers. This is the first evidence that influenza virus have been demonstrated from the patients with Reye's syndrome in Thailand. Our finding supports the previous reports from Europe and United States that the most likely cause of Reye's syndrome is viral in origin (Linnemann *et al.*, 1974; Cullity and Kakulas, 1970). However, the nature of the association between the viral infection and the clinical syndrome is unknown. It is possible that a previous exposure to toxin might be sufficient to potentiate or alter a normal viral infection, since aflatoxin has also been suggested a possible cause of

Reye's syndrome in children in Northeast of Thailand (Olson *et al.*, 1971). Such a virus/toxin interaction has been reported in laboratory animals (Colon and Sandberg, 1973). Because of the cases studied is still small and this is only a preliminary report of our finding, further investigations to explain this clinical syndrome is needed.

#### SUMMARY

Two cases of Reye's syndrome from Ramathibodi Hospital were reported. Both patients lived in the Bangkok area. They survived their illness after exchange blood transfusion. Influenza A virus was isolated from nasopharynx of case 1, and four-fold increases in hemagglutination inhibition titers of the same virus were also demonstrated in both cases.

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