

## Encephalopathy and Fatty Degeneration of the Viscera in Thai Children

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**OBJECTIVE :** A systematic study of the epidemiology, etiology, pathology and clinical features of Encephalopathy and Fatty Degeneration of the Viscera in Thai Children (EFDV).

**DESCRIPTION :** From 1 January 1969 until 31 December 1969, epidemiologic, clinical and pathologic data were collected on all patients admitted to the Udorn Provincial Hospital with diagnoses of encephalopathy, encephalitis or convulsions.

**PROGRESS :** A total of 109 patients were included in the study.

The diagnosis of "probable" EFDV was made if the following clinical features were present:

1. Sudden onset of convulsion or coma;
2. Serum glucose of less than 50 mg% (Folin-Wu) and/or cerebrospinal fluid glucose less than 40 mg%, and/or SGOT greater than 80 sigma units;
3. Absence of neutrophils and no more than 10 lymphocytes in the spinal fluid.

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A case was considered "definite" EFDV, if:

1. The above clinical criteria were present and a liver biopsy showed characteristic small vacuole fatty metamorphosis;
2. An autopsy revealed cerebral edema without cellular infiltration and a characteristic pattern of fatty metamorphosis in the liver, kidney, and heart.

Using the above criteria, 40 cases were diagnosed as "definite" EFDV (12 biopsies and 28 autopsies) and 27 were diagnosed as probable EFDV. Except for hypoglycemia, sixteen additional patients met all criteria for a diagnosis of probable EFDV; all of these patients had had IV glucose before the blood sugar determination.

#### Epidemiologic aspects

Of the 67 definite or probable cases, 38 were girls and 29 boys. All but six patients were between 18 months and 6 years of age. Forty-six (70%) of the patients died.

All but four of the patients came from rural areas. Since 10% of the population of Udorn province lives in the city of Udorn, and the rural population has much less ready access to the hospital than those in the city, the predominately rural distribution of this disease is emphasized. Two patients were siblings and immediate neighbors of a third child admitted two weeks earlier. In two other instances two children were from the same village. These were the only instances of clusters in the geographic origin of cases. However, 33 of all 162 siblings (20%) of patients, and 26 of the 58 between 18 months and six years of age, were ill within two weeks of their siblings' illness. In most instances this consisted of non-specific symptoms such as fever, URI's and/or headache.

#### Clinical syndrome

In nine of the 67 patients there was no history of symptoms prior to the onset of convulsions and coma. In the other 58 such prodromal symptoms tended to be mild and in most were less than 24 hours in duration. These included symptoms of URI, fever, headache, diarrhea, abdominal pain and vomiting.

In most patients, then, the onset of symptoms indicating serious disturbances of central nervous system function were unexpected and acute. Each of the 67 patients was brought to the hospital because of convulsions and coma. One patient died a few minutes after admission. Of the other 66, 54% were febrile at the time of admission, 46% had abnormal respiratory patterns (tachypnea, hyperpnea, Cheyne-Stokes, etc), and 23% showed hepatomegaly. Only three patients were jaundiced, and three others had gross irregularities in cardiac rhythm. All patients appeared well-nourished and in no patient was a skin rash present.

All but one patient was semi-comatose or comatose on admission, and 43 patients exhibited seizures. Thirty-one of these were in status epilepticus. Twenty-three of the patients displayed intermittent decerebrate posturing. Only one patient showed signs of meningeal irritation.

In all patients symptoms evolved acutely despite therapeutic attempts to correct hypoglycemia and electrolyte abnormalities and control cerebral edema. Thus 25% of the 46 fatal cases died within twelve hours of the onset of CNS symptoms, and 61% within 24 hours. No patient died later than three days after onset. Conversely, survivors generally had improved markedly within 48 hours of hospitalization. Only two patients still showed significant residual effects upon discharge, and both of these continued to improve thereafter. The clinical syndrome presented by these patients was remarkably constant. In most instances the diagnosis could be correctly made on the basis of history and physical examination, and in all cases where autopsy subsequently established a tissue diagnosis, the clinician had correctly diagnosed whether the patient had EFDV syndrome or not.

A summary of laboratory data is shown in Table 1.

LABORATORY RESULTS

A = Autopsy - Proven  
 B = Biopsy - Proven  
 C = Clinically Diagnosed

TEST	NO.	RANGE	MEDIAN	MEAN	STD. DEV.	COMMENTS
<b>Glucose</b>						
A	23	12 - 90	30	32.8	18.7	
B	8	13 - 65	31.5	27.1	16.2	
C	27	0 - 214	26.9	39.3	37.5	
<b>SGOT</b>						
A	23	50 - 1900	112	—	—	74% > 75
B	9	78 - 1250	250	—	—	100% > 75
C	26	120 - 156	133.5	133.2	—	100% > 75
<b>SGPT</b>						
A	23	16 - 875	69	—	—	70% > 40
B	9	30 - 1000	75	—	—	78% > 40
C	26	32 - 75	48.3	49.3	—	2% > 409
<b>Total Bilirubin</b>						
A	21	0.0 - 9.2	0.5	—	—	19% ≥ 2.0
B	9	0.0 - 3.6	0.8	—	—	22% ≥ 2.0
C	25	0.0 - 8.9	0.3	—	—	16% ≥ 2.0
<b>Prothrombin Time</b>						
A	7	24 - 40	33	32.0	—	86% ≥ 25
B	10	17 - 83	20.5	28.8	—	20% ≥ 25
C	11	15 - 35	22	21.9	—	18% ≥ 25
<b>CO<sub>2</sub> Content</b>						
A	18	7.4 - 20.9	16.9	15.6	—	83% < 19
B	12	10.8 - 26.5	13.8	15.9	—	75% < 19
C	24	8.9 - 26.0	18.0	16.2	—	75% < 19
<b>Chloride</b>						
A	26	80.5 - 122	101.5	100.7	—	38% < 98
B	12	91.5 - 108	101.5	101.0	—	17% < 98
C	26	75 - 105.8	96.1	97.6	—	50% < 98
<b>Potassium</b>						
A	24	4.1 - 7.4	5.8	5.8	—	58% > 5.6
B	10	3.2 - 7.4	4.7	4.8	—	10% > 5.6
C	26	3.2 - 7.5	4.8	4.9	—	12% > 5.6
<b>Sodium</b>						
A	24	116 - 158	131.5	132.8	—	50% < 132
B	10	120 - 160	137.0	138.9	—	20% < 132
C	26	120 - 156	132.5	133.3	—	42% < 132
<b>Hematocrit (%)</b>						
A	20	28 - 44	36	35.6	—	20% < 32
B	12	19 - 43	36	34.2	—	17% < 32
C	23	26 - 42	34	34.8	—	22% < 32

TEST	NO.	RANGE	MEDIAN	MEAN	STD. DEV.	COMMENTS
PTT						
A	7	57-166	78	89.9	—	3/7 > 80
B	10	40-106	59	61.3	—	1/10 > 80
C	11	44-489	72	115.7	—	4/11 > 80
WBC						
A	19	900-23,100	12,100	12,100	—	53% > 11,000
B	12	4,000-33,300	13,000	15,600	—	58% > 11,000
C	23	5,200-48,500	15,100	16,300	—	70% > 11,000
Polys (%)						
A	16	13-87	69.5	—	—	
B	12	27-92	69	—	—	
C	23	37-90	73	—	—	
Lymphs (%)						
A	16	15-87	28.5	—	—	
B	12	7-64	28.5	—	—	
C	23	10-61	27	—	—	
Total Protein						
A	13	5.7-7.5	6.3	6.6	—	
B	5	6.1-7.5	6.4	6.6	—	
C	15	6.0-7.7	6.8	6.8	—	

#### Pathology

A detailed report of the pathologic findings is now in preparation. The availability of more case material and fresh biopsy specimens have provided the following new observations:

1. There is ten to fifteen per cent enlargement of hepatocytes and hepatocyte nuclei;
2. Markedly enlarged irregular hepatocyte nucleoli are present in most cases;
3. Diffuse lymphocytolysis is a constant finding;
4. Neuronal degeneration is commonly found.

#### Lipid analyses

Specimens of liver and kidney were homogenized in a Waring blender, lyophilized, weighed and extracted three times with chloroform:methanol according to the method of Folch. The tissue was re-lyophilized, reweighed and the extractable lipid expressed as milligrams per gram of lyophilized tissue. The chloroform:methanol extracts were evaporated to dryness and then brought up to a volume of twenty-five cc. in chloroform. For chromatographic studies, an aliquot was adjusted with chloroform so that ten cc. of sample represented one gram of lyophilized tissue. Thin layer chromatography was performed using commercially available sheets (Eastman Chromagram System) according to the manufacturer's instructions. Identification of the various lipid fractions was accomplished by using known standards, published Rf values and spot tests.

Chemical analyses for total lipid, cholesterol, triglycerides, free fatty acids and phospholipids were performed on each extract. Two samples were taken from each tissue specimen and 2 replicate analyses were performed on each extract.

Lipid analyses were performed on liver and kidney specimens from 7 "controls" (patients dying with disease other than EFDV) and on 17 patients dying with EFDV. The average weight of lipid extractable from one gram of lyophilized liver and kidney tissue from "control" patients were 232 mg. and 199 mg. respectively; for patients with EFDV, the values were 558 and 349 respectively. Chromatographic analyses of the lipid revealed a marked increase in triglycerides, diglycerides and free fatty acids in the liver, with only triglycerides being increased in the kidney. Chemical analyses of the hepatic lipids revealed:

Result (Per. Gm. lyophilized tissue)	"Control"	Patient
Free Fatty Acids (mEq.)	0.138	0.276
Phospholipid (mg.)	52.0	39.0
Triglycerides (mg.)	10.7	109.5
Cholesterol (mg.)	11.5	9.0
Total Lipid (mg.)	212.0	550.0

Results of the renal lipid analyses are not yet completed.

#### Viral studies

Specimens of brain, liver, heart, kidney, and lung were collected under aseptic conditions and stored at  $-60^{\circ}\text{C}$  in individual, sterile, plastic bags.

After thawing rapidly, a 20% suspension was made by grinding with sterile sand in Hanks balanced salt solution, the suspension was then clarified by centrifugation, and inoculated into suckling mice and primary monolayer cultures of rhesus monkey kidney, Hela and WI-38 cells. Some specimens were also inoculated into primary human embryonic kidney cells. Thereafter all systems were observed for evidence of virus, i.e., illness in suckling mice, cytopathic effects, hemadsorption, or challenge virus resistance (using echo virus type 11 in monkey and human kidney cells.)

Serological tests were limited to hemagglutination-inhibition against arbovirus group A and B antigens. Paired sera (on admission and at least two weeks later) collected from survivors with documented disease were extracted with acetone and tested against dengue types 1-4, Japanese B encephalitis, and Chikungunya virus.

A total of 89 specimens from 26 autopsies were cultured for virus. These included tissues from 26 brain specimens, 20 lungs, 11 livers, 10 kidneys, 8 spleens, 7 hearts, 5 nodes, one thymus and one acute serum. All specimens were negative for a transmissible agent. A total of eleven paired sera obtained during the acute illness and at least two weeks later were tested for hemagglutination-inhibiting antibodies to dengue types 1-4, Japanese B encephalitis and Chikungunya viruses. None showed evidence of recent infection with these agents.

#### Acute toxicity of aflatoxin B<sub>1</sub> in the monkey

Further analyses of the mycotoxin obtained from the left over food samples revealed the toxin to be Aflatoxin B<sub>1</sub> (see 1969 Annual Report). This was confirmed by ultraviolet, infrared, mass spectroscopic and nuclear magnetic resonance analyses (courtesy of Dr. George Buchi, Mass. Inst.).

In order to establish the LD<sub>50</sub> of this toxin in monkeys and to observe the biochemical and pathologic responses to acute toxicity, the following experiment was carried out.

Five groups of four female macaques were given a oral single dose of 0.5, 1.5, 4.5, 13.5 or 40.5 mg/kg of chromatographically pure, crystalline aflatoxin B<sub>1</sub>. The toxin was prepared from the Aspergillus flavus grown from the B. kota food sample. A control group of 5 animals received no toxin. All animals receiving 40.5 and 13.5 mg/kg and one animal receiving 4.5 mg/kg died.

All 9 of the animals that died became drowsy 2-3 days after toxin administration; some experienced vomiting and/or convulsion; all these animals showed grossly fatty livers and kidneys with little other macroscopic changes. Hepatic cell necrosis was pronounced in the animals receiving the higher doses. Fatty degeneration of the hepatocytes, renal tubular epithelium and myocardium were constant findings. Similar, but less severe, changes were seen in tissues from the surviving monkeys (sacrificed at the end of 7 days).

The LD<sub>50</sub> for aflatoxin B<sub>1</sub> in the monkey was calculated for the test period of 6 days according to the method of Litchfield and Wilcoxon. The LD<sub>50</sub> is 7.8 mg/kg body weight with 95% confidence limits of 3.5–17.6 mg/kg body weight; the slope function of the LD<sub>50</sub> plot is 2.25.

Serum chemical analyses revealed that by day 3 after ingestion of toxin all the gross changes in test values had occurred in those that either went on to die or recovered. By day 1 some of the animals that later showed disease had early changes.

The following tests showed no evidence of significant alterations (compared to controls or their respective dose groups) over these 3 days:

Cholesterol  
 Total Protein  
 Electrolytes (Na, K, Cl)  
 Gamma Globulin  
 A/G Ratio

The following tests showed changes at higher dose levels but were difficult to analyze because of the wide variability of values for control and test animals and the absence of day 0 values:

Total Lipids (depressed)  
 Triglycerides (increased)  
 SGOT, SGPT (increased)  
 Alpha 2 and Beta Globulin (depressed)

Serum glucose and nonesterified fatty acids were the most interesting tests. Both showed changes (glucose decreasing, NEFA rising) that appear to be dose-related. But, whereas NEFA increases (as well as phospholipid decreases) were noted on day 1, values for several animals in the 3 high dose groups the glucose levels were still stable (see Table I):

Table I. Mean values on days 1 and 3 for serum glucose, NEFA and phospholipid determinations.

Dose Mg/Kg BW (4 monkeys/group)	Glucose (Mg%)		NEFA (Mg/L)		Phospholipids (Mg%)	
	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3
Controls	93.0	67.0	.602	.910	161.2	158.1
0.5	97.2	76.0	.657	1.090	183.8	198.2
1.5	72.5	64.8	.530	.825	190.0	181.9
4.5	64.8	50.0	1.080	1.665	140.6	146.9
13.5	83.5	35.0	.810	2.268	103.1	121.9
40.5	87.8	15.5	.740	2.535	100.8	126.2

The fairly large variation between days for these glucose and NEFA levels makes this observation harder to substantiate. But analysis of the paired glucose—NEFA values by animal revealed a nonsignificant correlation coefficient ( $r = -.257$ ) for day 1 but a highly significant value ( $r = -.906$ ) for day 3 values. This substantiates the definite changes on day 3, whereas any day 1 changes were not yet concurrently present. A closer look at the paired values on day 3 reveals 2 populations of results, both fairly normal or both very abnormal, with no intermediate values seen. Thus, even though a graded dose—response relationship is present for each test, values of both tests hardly overlap. This is also true for the survivors versus dead groups regardless of dose (Table II).

Table II. Comparison of Day 3 NEFA and GLUCOSE values in survivors and those that later died.

Test		Died n=9	Survived n=11
NEFA	RANGE	1.40—3.19	0.62—1.57
GLUCOSE		8.5—60	60—91
NEFA	MEAN	2.458	1.037
GLUCOSE		23.60	68.64

Day 3 values were further analyzed by log dose—response regression procedures. The NEFA values were uncorrected; for the glucose values the variable used was day 3 value as a proportion of day 1 value to try to stabilize the sizeable mean differences within each dose group. Both curves had highly significant slopes, .903 for NEFA and  $-0.332$  for glucose, but a good linear fit was found only for the NEFA values. Phospholipids may likewise be changing early (Table I) but further analysis is not possible due to missing values thereafter.

The significance of these findings lies not only in being able to document the biochemical changes leading to the observed histologic damage but also in demonstrating graded responses by dose and early, though definitely abnormal, changes. The possibility of diagnosing less severe and non—fatal disease (? pathogenesis dose—dependent) opens up many optimistic areas for investigation.

#### Aflatoxin assay

Quantitative chemical analysis for aflatoxins were performed on freshly frozen autopsy tissues, using a slightly modified version of the method of Eppley. Confirmation of identity was made by the chemical derivatives method of Andrellous and Reid. Results of analyses of tissue specimen are given below. Analyses were also performed on 7 non—EFDV patients.

AFLATOXIN ANALYSES  
Non EFDV Patients

Diagnosis	Age	Sex	Brain	Liver	Kidney	Stool	Stomach Constents	Intestinal Contents
Auto Accident	17	M	$\frac{2}{T}$	—	$\frac{2}{T}$	—	—	—
Auto Accident	20	M	ND	—	ND	—	—	—
Diphtheria	$1\frac{1}{2}$	F	T	ND	—	ND	—	T
Diphtheria	1	F	3	3	—	—	—	—
Pneumonia	2	M	3	3	T	—	—	—
Miliary T.B.	4	M	—	T	ND	—	—	—
Nasopharyngeal abscess	2	M	—	T	2	—	—	—

AFLATOXIN ANALYSES  
EFDV patients

Case Number	Age	Sex	Brain	Liver	Kidney	Stool	Stomach Contents	Intestinal Contents
UA-5 (009)	11/2	F	T	T	—	—	T	—
UA-9 (013)	3	M	—	T	—	T	T	—
UA-18 (028)	2	F	—	T	T	T	ND	—
UA-20 (032)	4	F	T	—	T	ND	T	—
UA-24 (035)	6	F	3	3	3	123/15	T	—
UA-25 (038)	2	M	2	3	—	ND	T	—
UA-26 (039)	12	M	ND	ND	—	ND	ND	—
UA-27 (040)	4	F	2	2	4	ND	T	—
UA-28 (041)	7	M	3	3	—	108/19	T	—
UA-29 (042)	3	F	3	3	ND	ND	5/2	—
UA-31 (045)	2	M	—	—	4	—	—	—
UA-32 (049)	12	M	ND	3	—	—	127/15	81/10
UA-34 (053)	10	F	2	2	1/T	—	—	—
UA-35 (056)	6	F	2/T	2	—	ND	116/19	—
UA-36 (057)	5	F	ND	ND	ND	13/4	ND	—
UA-38 (061)	10	F	ND	T	ND	ND	ND	—
UA-39 (074)	13	M	ND	47/6	3	T	11	—
UA-41 (082)	2	M	—	—	—	ND	2	—
UA-44 (101)	3	M	3	2	7	ND	ND	ND
UA-46 (103)	2	F	T	T	T	—	—	—
UA-47 (105)	18/12	F	—	—	—	ND	ND	T

**SUMMARY:** Between 1 January and 31 December 109 cases of possible encephalopathy and fatty degeneration of the viscera (EFDV) were studied at the Udorn Provincial Hospital. A diagnosis of either "definite" or "probable" EFDV was made in 67 cases. Clinical, laboratory and pathologic data was collected and analyzed. Viral studies on 89 specimens from 26 cases showed no evidence of viral infection. Tissue lipid analyses revealed the increased hepatic lipids to be predominately triglycerides and free fatty acids. Aflatoxin assays on autopsy specimens were positive in over 90% of cases. The LD<sub>50</sub> for aflatoxin B<sub>1</sub> in the macaques was established as 7.8 mg/kg body weight. Animals receiving a lethal dose of the toxin developed clinical and pathologic findings similar to patients with EFDV.

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