Animal Models for Reye's Syndrome

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1. Introduction

1.1. Historical Background

Reye's Syndrome (RS) was first documented by Douglas Reye and colleagues in 1963 as an "encephalopathy with fatty degeneration of the viscera," a rare entity, but one that at that time was associated with an 80% mortality rate (Reye et al., 1963). Although the apparent incidence and outcome of the disease have improved, it continues to be one of the major causes of noninflammatory neurologic death after a viral illness in children (Sullivan-Boylai and Corey, 1981; Heubi et al., 1987).

1.2. General Features of the Human Disease

Reye's Syndrome is defined clinically as a nonspecific encephalopathy with fatty visceromegaly that has a biphasic course. The first phase of the disease is typically a mild, unremarkable viral illness, frequently influenza B or varicella. The second phase begins when the child is beginning to recover from the viral illness, and is associated with profuse and persistent vomiting. Behavioral changes, including lethargy, confusion, and combativeness can rapidly (within 4–60 h) progress to stupor, coma, and death.

Morphological and biochemical findings have led many to speculate that the primary insult in RS is an acute, self-limiting disturbance in hepatic mitochondrial structure and function (La

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Montagne, 1983; Mowat, 1983). There is a characteristic reduction in the numbers of liver mitochondria. Those present are swollen dramatically with ultrastructural loss of their matrices and cristae (Daughtery et al., 1987). However, there is no evidence of cellular necrosis, and the mitochondrial distortions are completely reversed upon recovery from the disease. The biochemical changes are also indicative of a mitochondrial disease, since the metabolic pathways that are affected (urea cycle, gluconeogenesis, fatty acid oxidation) are localized either in whole or part in the mitochondria (for review, *see* Brown and Forman, 1982). At least some products of the blockade of these pathways (e.g., ammonia, fatty acids) are potential neurotoxins, and have been implicated strongly in the encephalopathic phase of the disease. Serum ammonia levels are extremely high during the early course of the disease, and in some cases have been reported to be 60-fold higher than normal levels (Tang et al., 1975). Tonsgard (1989) recently reported that serum levels of both monocarboxylic and dicarboxylic fatty acids are elevated at certain stages of the disease. There is also evidence that the relative proportions of unesterified polyunsaturated fatty acids are increased in serum (Ogburn et al., 1982) and in erythrocytes (Schwarz et al., 1987) of RS patients. Contiguous with serum lipid abnormalities, the cells of the visceral organs, particularly the liver, are loaded with fat that is stored as microvesicular droplets (Brown and Forman, 1982). This may be closely associated with a loss of regulation of adipose tissue lipolysis (Kang et al., 1982) and an increased uptake of fatty acids by the liver. Abnormalities in carbohydrate metabolism have been inferred from amino acid profiles (Romshe et al., 1981) and depletion of hepatic glycogen (Brown and Forman, 1982). In spite of this generalized mitochondrial malfunction, cytosolic enzyme activity is normal in RS patients. Moreover, the hepatomegaly alone does not appear to explain the deaths of the children, as the liver is often recovering while the brain continues to deteriorate.

The Centers for Disease Control (CDC) have set the following clinical and pathological criteria for a diagnosis of RS:

1. Acute, noninflammatory encephalopathy with cerebral spinal fluid (CSF) containing less than eight white blood cells/

- mm³ and histological sections of the brain demonstrating cerebral edema without meningeal or perivascular inflammation;
2. Fatty metamorphosis of the liver by biopsy or necropsy;
  3. Serum glutamic oxaloacetic transaminase (SGOT) levels greater than 3X normal, or blood ammonia levels greater than 1.5X normal; and
  4. No other explanation for the neurologic or hepatic abnormalities.

Gauthier et al. (1989) have suggested that the diagnostic criteria of the CDC be reassessed, as it is now believed that Reye's Syndrome may actually be two distinct disease entities, with the infantile form (i.e., in children less than 2–3 yr of age) attributable to the unmasking of an inborn error of metabolism, a group of diseases that include the organic acidemias, urea cycle defects, and medium-chain acyl-CoA dehydrogenase deficiency (MCAD) (Taubman et al., 1987; Greene et al., 1988; Roe, 1988). The "unmasking" may be a response to a specific illness, or to any disorder that limits caloric intake, and results in a disease that in many ways resembles RS. Factors that may distinguish inborn errors from RS include normal ammonia levels at presentation, a very short period between onset of initiating illness and pernicious vomiting, and lack of signs of increased intracranial pressure.

2. Encephalopathy of RS

2.1. Neuropathology

The encephalopathy of Reye's Syndrome is a noninflammatory, reversible process. Death, if it occurs, is usually owing to a severe brain edema in which the cerebral cortex has swollen to the extent that it becomes necrotic up against the nonyielding cranial vault (Venes et al., 1978). The swelling leads to increased intracranial pressure and altered cerebral perfusion (McWilliam and Stephenson, 1985; Frewen and Kisson, 1987). It is not known whether the edema is cytotoxic or vasogenic. The extensive histopathological studies of Partin et al. (1978) and others (Venes et al., 1978) have shown that there is an increase in

the number and size of astrocytes, and that they are often devoid of glycogen granules. Of particular interest is the fact that astrocytic mitochondria are relatively normal in structure. Depending on the severity of the disease, neurons may also be enlarged, and in contrast to the astrocytes, may contain pale, enlarged, and pleomorphic mitochondria. Capillary endothelial cells appear to be normal, with normal-appearing mitochondria, which is consistent with evidence that the blood-brain barrier is intact (Brown and Forman, 1982). Demyelination does not appear to occur, although Partin et al. (1978) have reported the presence of watery blebs within the myelin sheaths.

2.2. Biochemical Abnormalities

Relatively little is known regarding the biochemical pathology of the brain in RS. There are no outstanding abnormalities in lipid accumulation or metabolism (Brown and Forman, 1982), and the activities of enzymes that are altered in the liver appear to be normal (Robinson et al., 1978). Simultaneous measurements of arterial and jugular vein ammonia levels indicate that the toxic metabolite is taken up in large quantities by the brain in RS patients. The excessive load of ammonia appears to be related to increased brain lactic acid production (for review, *see* DeLong and Glick, 1982). Faraj and coworkers (1984) have demonstrated hypercatecholaminemia in patients with RS, and suggested that this might contribute to the encephalopathy of the disease. A recent report also indicated that CSF levels of neuron-specific enolase (NSE), a prominent neuronal cytosolic enzyme that is released upon neuronal destruction, are elevated in RS (Nara et al., 1988).

3. The Etiology of RS: Theories

In spite of our increasing understanding of the metabolic abnormalities in RS, its etiopathogenesis remains obscure (Pranzatelli and De Vivo, 1987; Murphy et al., 1989). In the following brief discussion, the prominent theories regarding the etiology of RS can be divided into two categories: those pertaining to the initiation of the disease process, and those concerning the events related specifically to the development of encephalopathy.

One of the consistent features of RS is that its development is associated with an antecedent viral illness involving one of several specific infecting organisms. However, since Reye's original publication in 1963, many investigators and clinicians have believed that a toxin is involved in some manner in the initiation of the disease, based on the fact that its progression resembles an acute toxic event. A wide range of exogenous toxins have been shown to produce at least some of the clinical features of RS (Pranzatelli and De Vivo, 1987); however, no single xenobiotic toxin or groups of toxins have been shown unequivocally to be the causative agent(s) (Crocker, 1982,1988). Within the past several years, considerable attention has focused on salicylates as a causative agent, and declines in aspirin use and in the incidence of RS tend to support an association between the two. However, attempts to demonstrate salicylate-dependent potentiation of viral effects in animal models generally have been unsuccessful (*see* Section 4.5.2.). In the human disease, there is no obvious genetic predisposition, and in cases involving siblings, one might presume that the RS-like disorder is actually an inborn error of metabolism (*see* Section 1.2.). Many investigators, including ourselves, now believe that exposure of susceptible individuals to environmental chemicals, including salicylates, can result in an abnormal and potentially lethal response to what is normally a relatively benign virus infection (Crocker, 1982).

There are few who would dispute that RS is a mitochondrial disease in which there are dramatic alterations in metabolism that result in production of high levels of otherwise normal metabolites. One of the primary focuses in RS research has been to determine which, if any, of these metabolites are responsible for the distinct encephalopathy of the disease. The three groups of metabolites that have been deemed the most likely candidates include ammonia, free fatty acids, and short chain organic acids, each of which is known to produce neuropathy in humans when present in excessive amounts (DeLong and Glick, 1982). A broad discussion of this topic is beyond the scope of this review, but it should be mentioned that for each of these groups there is substantial evidence against their being the sole encephalopathic factors in RS. Nevertheless, several working hypotheses have

evolved from the knowledge that individuals with RS are in a highly exaggerated catabolic state, and these have provided the basis for the development of several of the animal models described in this chapter.

4. Animal Models of RS

It is believed that the only way we are going to elucidate the causes of the encephalopathy in RS is to determine the sequence of events that occurs during the pathogenic process. This question is virtually impossible to address in patients, since the initiating events, and many of their biochemical consequences, have taken place (and may have reverted to normal) before the disease is diagnosed. The only way we can hope to understand the process is through the development of animal models that mimic the disease.

Although a variety of different approaches has been used to develop a suitable animal model for RS, it is generally accepted that no single model fulfills all of the criteria of the human disease (De Vivo, 1984; Deshmukh, 1985; Heubi et al., 1987). In some cases, the models have not yet been characterized completely, whereas in others, there is an apparent inability to reproduce a specific effect. The latter may be owing to the transient nature of many of the biochemical/morphological changes associated with this multifactorial disease. In the following sections, we shall describe the experimental protocols for the most widely used animal models of RS. We shall also indicate briefly which of the features of the human disease they successfully mimic, and some of the problems associated with each. The models have been divided into three groups, based on whether they involve toxin alone, virus alone, or a combination of toxin and virus. Data regarding the serum and liver changes, and the encephalopathy that occurs in each of the models is presented in Tables 1–3.

4.1. Models Associated with Environmental Toxins

Many attempts to establish animal models of RS on the basis of exposure to an environmental chemical (with or without virus infection) have evolved from epidemiological findings,

some of which were regional in nature. A variety of xenobiotic substances have been tested (Colon et al., 1974; Crocker and Bagnell, 1981; Hug et al., 1981; Crocker, 1982), and the results have led to the establishment of models that involve exposure to aflatoxin (Bourgeois et al., 1971; Thurlow et al., 1980), 4-pentenol acid (Glasgow and Chase, 1975; Thayer, 1984; Hart et al., 1989), surfactants (Crocker et al., 1976; Rozee et al., 1978; Hug et al., 1981; Crocker et al., 1986), and salicylates (Linnemann et al., 1979; Deshmukh et al., 1982).

4.1.1. Aflatoxin

Aflatoxin B₁, a toxic metabolite of *Aspergillus flavus*, was suggested as a candidate in the pathogenesis of RS based on the high incidence of encephalopathy and fatty degeneration of the viscera in Thai children, and on the recognized presence of the toxigenic fungi in many local food products, including rice. Two animal models have been based on aflatoxin toxicity: the monkey model of Bourgeois and coworkers (1971), and the rat model of Thurlow and colleagues (1980).

The Bourgeois model involves the use of female, crab-eating macaque monkeys (*Macaca fascicularis*) between 36 and 44 mo of age (average body wt, 1.7 kg), that are maintained on a primate diet supplemented with fruit. For the experiment, the animals are divided into six groups (4 macaques/group), each of which is fed a different concentration (0–40.5 mg/kg) of aflatoxin B₁ in single-dose gelatin capsules. On the day the toxin is given, the animals are kept under constant observation and blood samples are collected at regular intervals. Bourgeois and coworkers reported that animals that receive ≥ 13.5 mg/kg begin to die 67 h following aflatoxin administration, and all are dead by 149 h.

The most consistent clinical finding with this model is vomiting, which occurs in all animals that die. The animals that receive fatal doses go through a period of lethargy and depression prior to coma; however, a relatively small proportion experience convulsions. The encephalopathy is associated with increased ratios of brain:body weights, cerebral edema, swelling and separation of the myelin sheath, gliosis, and neuronal degeneration. The latter is manifested by shrinkage and increased eosinophilia of the cytoplasm, pyknosis and distortion of the

Table 1
Serum Abnormalities in Animal Models and in Human Reye's Syndrome

	Hyperammonemia	Transaminases SGOT SGPT	Free fatty acidemia	Hypoglycemia	Amino acidemia	References
Human RS	+	↑↓→	±	±	+	Crocker, 1982 Brown and Forman, 1982
Animal models						
Aflatoxin monkeys	ND ¹	↑	+	+	ND	Bourgeois et al., 1971
4'-Pentenoate rats	+	↑,→	ND	± (fasting dependent)	+	Glasgow & Chase, 1975; Thayer, 1984; Hart et al., 1989
Margosa Oil rats	+	↑	ND	+	ND	Sinniah et al., 1985
Octanoate infusion rabbits	+	→	+	-	ND	Trauner, 1982 PUFA
Rabbits Concentrated FluB mice	ND	↑	ND	(-) ²	ND	Kang et al., 1982
Endotoxin rats	+	↑	+	?	ND	Davis, 1986
	+	→	+	-	ND	Yoder et al., 1985

Hyperammonemia/FluB						
Ferrets						
Arg Diet	+	↑	ND	+	- ¹	ND Deshmukh et al., 1982; Deshmukh et al., 1983 Deshmukh and Thomas, 1985
Arg Diet+ FluB	+	↑	ND	+	ND	ND
Arg Diet + FluB + ASA	++	↑↑	ND	+	ND	ND
Surfactant/FluB						
Mice						
Surfactant	+	ND	ND	-	ND	ND Crocker et al., 1986
Surfactant + FluB		+	ND	ND	-	ND

¹ ND, not done or not reported, ² ( ), moderate changes

Table 2
Hepatic Abnormalities in Animal Models and in Human Reye's Syndrome

	Fatty liver	Mitochondrial swelling and degeneration	Liver enzyme activities ¹					Glycogen depletion	Inflammation and necrosis	References
			OTC	CPS	MAO	CAT	GDH			
Human RS	+	+	↓	↓	↓	→	→	+	-	Crocker, 1982; Brown and Forman, 1982; Mitchell et al., 1985
Animal models										
Aflatoxin Monkeys	+	ND ²	ND	ND	ND	ND	ND	+	+	Bourgeois et al., 1971
Rats	ND	ND	↓	↓	ND	ND	ND	ND	+	Thurlow et al., 1980
4-Pentenoate Rats	+	±	ND	ND	ND	ND	↓	ND	-	Glasgow and Chase, 1984; Thayer, 1984; Hart et al., 1989
Margosa oil Rats	+	+	ND	ND	→	↓	→	+	-	Sinniah et al., 1985
Octanoate infusion Rabbits	+	+	ND	ND	ND	ND	ND	ND	-	Trauner, 1982
Concentrated FluB Mice	+	-	↓	ND	ND	ND	ND	(+)	-	Davis, 1986
Spontaneous RS? Mice	+	+	ND	ND	ND	ND	ND	+	-	Brownstein et al., 1984

Endotoxin	+	+	ND	ND	ND	ND	ND	+	+	Yoder et al., 1985
Rats	+	+	ND	ND	ND	ND	ND	+	-	
Hyperimmunemia/ FluB										
Ferrets										
Arg diet	+	ND	→	→	ND	ND	ND	ND	ND	Deshmukh et al., 1982,1983; Deshmukh and Thomas, 1985
Arg diet + FluB	+	ND	↓	→	ND	ND	ND	ND	ND	
Arg diet + FluB + ASA	+	+	↓	↓	ND	ND	ND	+	ND	
Surfactant/FluB										
Mice										
Toximul	-	-	→	ND	ND	ND	ND	ND	-	Crocker et al., 1986
Toximul + FluB	-	+	↓	ND	ND	ND	ND	ND	-	
Toximul + EMC	+	+	↓	ND	ND	ND	ND	ND	-	Taylor et al., 1979
Atlox + EMC	±	+	↓	→	ND	ND	ND	+	-	Hug et al., 1981

^aAbbreviations of enzyme activities: OTC, ornithine transcarbamylase; CPS, carbamyl phosphate synthetase; MAO, monoamine oxidase; CAT, catalase; GDH, glutamate dehydrogenase.

²ND, not done or not reported.

Hyperammonemia/FluB									
Ferrets									
Arg diet	+		+	+(CE) ²	ND	ND	ND	ND	Deshmukh et al, 1982;
Arg diet + FluB	+		+	+(CE)	ND	ND	ND	ND	Deshmukh and Thomas, 1985
Arg diet + FluB + ASA	+		+	+(CE)	ND	ND	ND	ND	
Surfactant/FluB									
Mice									
Toximul	-		-	ND	-	ND	ND	ND	Crocker et al, 1986
Toximul + FluB	±		±	ND	+	ND	ND	ND	

¹ND, not done or not reported; ² CE, choroid plexus epithelial cells.

nuclei, and satellitosis. Systemically, significant serum changes include hypoglycemia, increased free fatty acids and transaminase activities, and decreased phospholipids. There is fatty infiltration of the visceral organs, including the liver, kidneys, and heart.

The rat model described by Thurlow et al. (1980) involves administering a single dose of aflatoxin B₁ (3 mg/kg, in dimethylsulfoxide or saline) to male Fischer rats (120–150 g). The animals are killed 2 or 24 h after treatment. The only information available regarding the effects of this treatment is that at 24 h, there are significant reductions in activity of the hepatic enzymes, carbamyl phosphate synthetase (CPS) and ornithine transcarbamylase (OTC) (40 and 30% inhibition, respectively).

One of the main criticisms of the theory that aflatoxin plays a role in the etiology of RS is that investigators have failed to confirm the presence of high levels of the toxin in livers of many patients who presumably died of the disease (Rogan et al., 1985).

4.1.2. 4-Pentenoic Acid (*Hypoglycin*)

Jamaican Vomiting Sickness is an RS-like disease that is believed to be caused by ingestion of hypoglycin, a toxic component of unripe akee fruit (Tanaka et al., 1972). On the basis that the two diseases might share a common pathogenesis, an animal model was established using 4-pentenoic acid, a structural analog of hypoglycin (Glasgow and Chase, 1975; Thayer, 1984; Hart et al., 1989).

In the most recently described 4-pentenoic acid model (Hart et al., 1989), adult male Sprague-Dawley rats (200–300 g) are given ten sc injections over the course of 3 d with purified 4-pentenoic acid (12.5 mg/kg, neutralized to pH 7.4) (chronic phase). A final dose (200 mg/kg) (acute phase) is administered 30 min before killing the animals. This model has been varied by other investigators with respect to dosage, route of administration (e.g., sc vs ip), chronic vs acute treatment, and fed vs fasted animals. The variability in the results obtained may well reflect these differences.

Rats injected with 4-pentanoate develop signs of acute encephalopathy, as evidenced by excitability followed by lethargy, seizure activity, coma, and death. Generally, the animals rapidly become hyperammonemic, have elevated levels of

serum transaminases and amino acids, are hypoglycemic, and have fatty infiltration of the liver. The latter two abnormalities occur only in fasted animals, which is believed to reflect pentenoate-dependent inhibition of fatty-acid oxidation (Thayer, 1984). Rats that are given 4-pentenoate orally do not develop hepatic fat deposits or mitochondrial structural abnormalities (Thayer, 1984).

The features that most readily distinguish this animal model from human RS are the absence of serum fatty acidemia and the fact that the animals do not experience an antecedent illness.

4.1.3. Margosa Oil

Margosa oil (MO), an unrefined fatty acid-rich extract of the seeds of the Neem tree, was used for centuries as an oral home remedy for cough and general malaise throughout many Indo-Malaysian countries (Sinniah et al., 1981). Concern for its safety arose when it was found that its ingestion produced a syndrome in young children that had many of the features of RS, including severe brain edema, coma, and a high rate of mortality (Sinniah and Baskaran, 1981; Sinniah et al., 1982). The active component of the oil has not been identified, but there have been suggestions that it may be an organic sulphur compound (Sinniah et al., 1981) and/or aflatoxins B and G (Sinniah et al., 1982). Koga et al. (1987) recently suggested that the toxic effects of MO may involve one or more of the oil's long- or medium-chain fatty acids and/or the mitochondrial toxins nimbin or nimbiol. The unrefined oil is not available legally on a commercial basis, which restricts its widespread use for an animal model.

Sinniah and coworkers have studied the MO model of RS most extensively in the rat (Sinniah et al., 1985; Koga et al., 1987), although their initial studies were carried out with mice. In the former, male Sprague-Dawley rats (250 g) are given two ip injections of either corn oil (5 mL/kg) or MO (5 mL/kg), one at time 0, and the second 15 h later. This schedule was set up to mimic the sequence of events associated with development of Reye's-like Syndrome in children following ingestion of the oil. The animals are killed by cervical dislocation 18–20 h after the last injection.

Within 2 h of the first injection, the MO-treated animals exhibit tachypnea and lethargy. Just prior to the second dose, they become hyperreactive to a sudden noise, and shortly after they develop florid neurological signs, including hyporeactivity, coma, and seizures. However, there is no evidence of structural damage or cerebral edema, as judged by a normal brain water content. Serum analyses indicate hyperammonemia, hypoglycemia, and elevated aspartate and alanine transaminase activities. There is no indication in the literature as to whether MO alters serum free fatty acid levels or amino acid profiles. Microvesicular fat droplets are present in the liver and kidney, but not in the brain. There is no evidence of necrosis or inflammation in the liver; however, hepatic mitochondria are swollen, with loss of matrix, cristae, and dense bodies, and liver glycogen is depleted. Of the liver enzyme activities studied (monoamine oxidase, glutamate dehydrogenase, catalase, glucose-6-phosphate dehydrogenase, and lactate dehydrogenase), only catalase was reduced significantly. The results of recent *in vitro* studies indicate that mitochondrial oxidative phosphorylation is uncoupled by MO, that Stage 4 respiration is increased, whereas the respiratory control ratio is decreased, and that mitochondrial ATP content is reduced (Koga et al., 1987).

The two features of the MO model of RS that appear to be inconsistent with the human disease are the absence of cerebral edema (even though encephalopathy is present) and the relative lack of effect on hepatic mitochondrial activities.

4.2. Metabolic Toxin Models

Another approach that has been used to develop animal models for RS has been to bypass the "triggering insult" step and to expose the animals to metabolites that are purported to be the underlying causes of the encephalopathy. Octanoic acid and polyunsaturated fatty acids are the two metabolites that have been studied most extensively.

4.2.1. Octanoic Acid

Octanoic acid (C_8) is an eight-carbon, saturated fatty acid that is one of the metabolites formed during beta oxidation of longchain fatty acids. Based on observations that levels of

octanoate are significantly elevated in some children with RS (Mamunes et al., 1975; Trauner et al., 1975; Ogburn, 1976), Trauner and colleagues established a model in which they infuse the fatty acid into adult rabbits (Trauner and Huttenlocher, 1978; Trauner, 1981, 1982).

Male 2-kg rabbits are anesthetized with sodium pentobarbitol (30–50 mg/kg) and catheters are placed in the carotid artery and ear veins. When the animals wake up and their vital signs are stabilized, sodium octanoate (0.2M, pH 7.4) is infused via the ear vein for a 4 h period. During this time, blood is drawn hourly, and vital signs (heart and respiratory rates, blood pressure, electroencephalogram) are monitored continuously. The rabbits are killed after the 4-h infusion by a rapid iv injection of pentobarbitol (100 mg).

During the infusion of octanoic acid, the rabbits hyper-ventilate, develop hypotonia, coma, and papillary dilatation, and approx one-third have convulsions after 1 h of treatment. Serum changes include increases in ammonia (300%), SGOT (143%), lactic acid (183%), and octopamine (300%). Histopathological studies of the liver indicated that the infusion results in microvesicular fat accumulation without hepatocellular necrosis or inflammation, and that the only ultrastructural abnormalities are swollen, pale, and pleomorphic mitochondria that have "fluffy" matrices.

Trauner and coworkers did not find any evidence of generalized cerebral edema in the rabbits, although levels of brain octanoate were significantly elevated. However, they did report that there were signs of early cellular damage, including swelling and degeneration of brainstem neurons and occasional swelling of astrocytic foot processes. These investigators have also demonstrated that intracranial pressure is elevated in the animals (Trauner and Adams, 1981).

4.2.2. Polyunsaturated Fatty Acids

The rabbit model of Kang et al. (1982) is based on observations that serum from children with RS contains abnormally high levels of unesterified polyunsaturated fatty acids (PUFA) (Ogborn et al., 1982), and on the theory that the circulating PUFA could be related to the encephalopathy.

In this model, New Zealand White male rabbits (*Oryctolagus cuniculus*, 2–4 kg) are anesthetized (halothane/O₂) and placed in a prone position on a table with the head flexed over the edge at right angles to the spinal column. With mature animals, a 25 gage, 16 mm needle is inserted into the cisterna magna until CSF fills the needle. Suspensions of the fatty acids (0.1–0.2 mL) are injected using a tuberculin syringe attached to the needle. Following injection, the animals are placed in a head-down position for 1 min, and then placed on a flat surface for observation. For injection, the fatty acids are suspended in normal rabbit serum/isotonic saline (1/9, v/v) containing 600–1200 µg protein. Kang and coworkers used 11,14-eicosadienoic acid (C20:2ω6) for many of their experiments. In some studies, they administered the bee venom peptide, mellitin, to stimulate phospholipase A₂ activity and release fatty acids from endogenous lipids.

Increasing degrees of neurological abnormalities develop in a dose-dependent manner consequent to intercisternal administration of free unsaturated fatty acids. Relatively mild, early changes (Level 1) include hyperventilation, lethargy, and diminished spontaneous movement. A second stage (Level 2) involves an increase in the rate and depth of respiration; the animals are very hyperactive, and in some cases, have tonic-clonic movements of the extremities. This behavior is more exaggerated at higher doses (Level 3), and further increases result in convulsions and coma within 10 min after the injection (Level 4). Fatal cases proceed to a state of apnea (Level 5). Kang et al. demonstrated that encephalopathy did not develop with administration of saturated fatty acids, and that the most profound neurological responses were produced by arachidonic (C20:4ω6) and docosahexaenoic (C22:6ω3) acids, two PUFA that are present in high concentrations in the brain. Injection of melittin into the cisterna magna, and infusion of PUFA into the internal carotid artery produced effects similar to those with intercisternally administered PUFA, however, systemic administration of the acids produced only mild hyperventilation. Interestingly, pretreatment of the rabbits with aspirin reduced the severity of the neurotoxic effects of the PUFA.

4.3. Bacterial Endotoxin Model

One of the most recently developed animal models for RS involves administration of low doses of endotoxin to rats, with or without low doses of aspirin (Yoder et al., 1985; Kilpatrick et al., 1989). The model is based on observations that serum from RS patients contains elevated levels of these toxins and that these substances stimulate production and release of bioactive compounds (e.g., monokines) that are responsible for the *in vivo* toxic effects (e.g., activation of lysosomal proteases).

In the model, male Sprague-Dawley rats (250–300 g) are fasted for 12 h, at which time they are given *ip* injections of either placebo (5% dextrose in water) (controls) or a sublethal dose (0.2 mg/kg) of *E.coli* 0111:B4 lipopolysaccharide (LPS) (Sigma Chemical Co., St. Louis, MO). The animals are fasted for an additional 12 h, at which time they are killed. Blood samples are taken at 0, 12, and 24 h for determination of baseline, preinjection, and final levels of ammonia, fatty acids and transaminase activities.

Although this model has not been extensively characterized, there is evidence that it reproduces several of the hallmark features of RS. Although Yoder et al. report that none of the animals died before completion of the study, they became lethargic, ataxic, and had decreased self-grooming behavior by 4 h post LPS injection. Brain examination indicated an absence of necrosis and inflammation; microscopic and ultrastructural analyses were normal, with the exception of some proliferation of Type II astrocytes in 2/10 animals. Serum analyses indicated that endotoxin administration was associated with significant (approx twofold) elevations in ammonia, free fatty acids, and lactic acid; fasting alone did not affect these parameters. Unlike the situation in RS patients, serum glucose was normal, as was SGOT activity. Examination of the livers indicated that the endotoxin-treated rats had appreciably greater fat deposition than their fasted controls, and that the mitochondria from 6/10 of the LPS animals were pleomorphic, with expanded and disrupted matrices. In some cases, these changes included swollen mitochondrial cristae and proliferation of endoplasmic reticu-

lum. The remaining four rats in this treatment group had less prominent mitochondrial damage. Endotoxin treatment also resulted in liver glycogen depletion.

In their more recent studies, Kilpatrick et al. (1989) have added acetylsalicylic acid (ASA, aspirin) to their experimental paradigm. One hour after the injections of either placebo or LPS, half of each of the two groups is given an ip injection of dextrose and the other half receive ip injections of ASA (50 mg/kg). The results of these studies indicate that addition of ASA results in hepatic production of high levels of the unusual acyl-CoA esters and branched-chain amino acid metabolites that have been reported to be present in RS patients. These observations have led to the proposal that one way in which ASA could be involved in RS is by exacerbating endotoxin-dependent production of toxic metabolites.

4.4. Virus Models

4.4.1. Concentrated Influenza B Virus

The most widely studied animal model that employs virus alone is that of Davis and coworkers (Davis et al., 1983; Davis and Kornfeld, 1986; Davis, 1987; Trauner et al., 1988). In this model, juvenile (3–4-wk-old) BALB/c mice (10–12 g) are injected iv with high concentrations of influenza B virus (Influenza B/Lee/40, American Type Culture Collection, Rockville, MD). The virus is an egg-adapted strain of influenza B/Lee/40 that is grown in embryonated eggs and concentrated by ultracentrifugation (Davis et al., 1983). It cannot be administered intranasally, since the high concentrations used will produce a fatal pulmonary congestion via this route. In the model, each animal is inoculated once with 6400 hemagglutination units (in 0.5 mL) through a tail vein. Davis and coworkers have added salicylates to the experimental design in some studies (Davis et al., 1985; Trauner et al., 1988), and the results of these are referred to in Section 4.5.2.

Thirty six to forty eight h after injection with the concentrated virus, the mice become ataxic and lethargic, have seizures spontaneously or if they are stimulated, and become comatose. Death occurs in 50% of the mice within 1–3 d following inocula-

tion. Laboratory findings indicate significant increases in serum ammonia levels and transaminase (SGOT) activity. Cerebrospinal fluid and serum bilirubin are normal (Davis et al., 1983). The investigators report that serum free fatty acids are elevated and glucose may be reduced, but no data are given (Davis and Kornfeld, 1986). There is an accumulation of microvesicular fat droplets in the liver, but one of the most significant features is that few, if any, changes are seen in hepatic mitochondrial structure. Injection of the virus results in a 60% inhibition of [^{14}C]palmitate oxidation by the isolated mitochondria (Trauner et al., 1988). Relatively little has been reported regarding the encephalopathy, other than a suggestion that some of the mice have evidence of mild cerebral edema without inflammation. Recent studies have indicated the presence of viral antigens within brain capillary endothelial cells, which suggests that this is a nonpermissive cerebral infection (Davis, 1987).

Several concerns have been raised regarding the Davis model for RS, two of which are the requirement for a very large viral inoculum and the fact that it is administered by an intravenous rather than the natural respiratory route. In terms of the laboratory findings, the major disadvantage of the model is that it does not reproduce the mitochondrial structural changes that are the hallmark of human RS.

4.4.2. Spontaneously Occurring RS?

In 1984, Brownstein and colleagues reported that on five separate occasions, a spontaneous, RS-like illness broke out among their colony of Balb/cByJ mice after the animals were exposed to indigenous murine viruses. Intestinal coronavirus was the pathogen most frequently associated with the acute illness. The mice that died following a rapid deterioration in consciousness had many pathologic findings (e.g., fatty steatosis of the liver, swollen mitochondria, elevated serum ammonia) characteristic of RS. Neuropathological assessment by light microscopy indicated that there was a significant increase in the number of astrocytes in specific brain regions, and that these had swollen, pallorous nuclei reminiscent of Alzheimer Type II astrocytosis. These changes are not seen in RS. Under electron

microscopy, the cytoplasm of cortical astrocytes was electron lucent and expanded, particularly in the pericapillary end-feet. There was evidence of lipid droplets and membrane-bound vacuoles, and a reduced number of glycogen particles. Astrocytic mitochondria were normal in structure. By contrast, neuronal mitochondria were swollen and contained rarified matrices and disorganized and fragmented cristae. With the exception of the Alzheimer type II astrocytic changes, these findings closely parallel those seen in the acute phase of human RS.

The Brownstein model raised the interesting question as to whether RS is an aberrant but spontaneous response to a viral infection. Unfortunately, it has not been possible to investigate this possibility further, since the investigators have not been able to reproduce the model.

4.5. Models Based on Chemical/Viral Interactions

4.5.1. Hyperammonemia and Influenza B

Deshmukh and coworkers established a ferret model for RS on the basis that this species readily becomes hyperammonemic on an arginine-free (Arg) diet, and is very sensitive to infection by human influenza viruses (Deshmukh et al., 1982). The investigators have shown that the combination of these two treatments produces some of the metabolic disturbances characteristic of human RS, and that the effects are exacerbated when salicylates are added to the regimen (Deshmukh et al., 1982, 1983; Deshmukh and Thomas, 1985).

A typical experiment (Deshmukh and Thomas, 1985) involves eight groups of young (8-wk-old) male ferrets (400–450 g) (Marshall Research Animals, Inc., North Rose, NY). On day 1 (10:00 am), groups 1, 4, 5, and 7 are inoculated intranasally under light ether anesthetic with human influenza B (Flu B) virus (Georgia/10179; viral titer, 10^6 EID₅₀ mL; 0.5 mL/naris). Acetylsalicylic acid (ASA, Sigma Chemical Co., St. Louis, MO, 50 mg/kg, twice daily) is given to groups 2, 4, 6, and 7 by feeding tube beginning on the evening of day 1 and continuing through the morning of day 4. On day 3 (1:00 pm) food is removed from all cages, and between 9:00 and 11:00 am on day 4, the control groups

are given cat chow, whereas those animals in groups 3, 5, 6, and 7 are fed the Arg diet. The latter diet contains amino acids, vitamins, a salt mixture, carbohydrates, and chicken fat. Arginine is replaced by an isonitrogenous amount of alanine. The animals in group 8 are maintained as controls.

Ferrets infected with human influenza B virus exhibit symptoms consistent with flu, including fever, lethargy, and sneezing. These animals do not become hyperammonemic or encephalopathic, even if salicylate is included in the experimental protocol (*see* Tables 1–4 for data summary). By contrast, ferrets that are fed an Arg diet become hyperactive within 2 h, and then progress rapidly to a comatose state. The encephalopathy is associated with 15–20-fold increases in serum ammonia levels. The encephalopathy and biochemical changes are most pronounced when the animals are given the combined FluB, ASA, and Arg diet treatment. In addition to the abnormalities just described, laboratory investigations indicate increased serum levels of free fatty acids and transaminases, normal bilirubin, increased prothrombin time, decreased liver ornithine and carbamyl transferase activities, and increased liver lipids and CSF levels of ammonia and glutamine. Electron microscopic analysis indicates swollen and pleomorphic liver mitochondria and decreased glycogen (Deshmukh, 1985). Recent studies have shown that there is intercellular vacuolization and edema in epithelial cells of the choroid plexus (Rarey et al., 1987).

Much of the data obtained in the studies of Deshmukh and coworkers suggest that the common denominator in the ferret model is hyperammonemia, and Heubi et al. (1987) have suggested that it is more representative of extreme hyperammonemia than of RS. However, as Deshmukh et al. have shown, elevations in serum ammonia levels per se (*i.e.*, by administration of jack bean urease) does not produce an encephalopathic state (Deshmukh et al., 1982).

4.5.2. Salicylate Potentiation of Viral Infections

Much of the recent publicity surrounding RS has centered around its purported connection with aspirin (acetylsalicylic acid; ASA). Although such a relationship tends to be supported by

apparent reductions in the incidence of the disease concomitant with reduced aspirin usage (Hurwitz, 1988), a causal relationship between the two has not been firmly established.

Salicylates were suggested to be likely candidates for involvement in RS, based on epidemiological studies and on the basis that ASA intoxication can mimic certain features of the syndrome, including hepatic abnormalities (e.g., acid-base disturbances, increased transaminase levels), hypoglycemia, and severe encephalopathy with cerebral edema (Quint and Allman, 1984). However, salicylism is not usually associated with the excessive elevations in blood ammonia concentrations that one sees in RS (De Vivo, 1984), or with increased serum amino acid levels (Quint and Allman, 1984). Moreover, Daugherty and coworkers (1987) have recently reported that unlike the case in RS, hepatic mitochondrial size and number are normal or near-normal in patients with salicylate poisoning.

Several groups of investigators have examined the effects of salicylates in their animal models of RS (Linnemann et al., 1979; Hug et al., 1981; Deshmukh et al., 1982, 1983; Davis et al., 1985; Rarey et al., 1987; Trauner et al., 1988; Hart et al., 1989). A summary of the results of these studies is presented in Table 4.

4.5.3. Surfactant-Dependent Potentiation of a Virus-Initiated Disease

In the early 1970s, investigators from our laboratories established a mouse model for RS based on observations that children who developed RS in the Maritime provinces were usually from rural areas in which active forestry spray programs were being carried out (Crocker and Ozere, 1979). Initial studies of the effects of exposing young animals to pesticides (and/or vehicle) and then infecting them with virus demonstrated that it was the vehicle, an industrial emulsifier, rather than the pesticide, that was active in potentiating viral lethality (Crocker et al., 1974; Taylor et al., 1979). We have now studied chemical potentiation of viral infection using several variations of this basic paradigm. The most recent and most extensively studied is a model in which neonatal mice are exposed dermally to nontoxic amounts of the emulsifier, Toximul MP8, and subsequently

Table 4
Effects of Salicylates in Animal Models of Reye's Syndrome

Experimental model	Serum $\uparrow$ NH ₃	Elevated transaminases	Fat	Liver		Encephalopathy		References
				mitochondrial abnormalities	Enzymes OTC CPS	Behavior changes	Mortality rate	
Ferrets								
ASA (50 mg/kg, b.i.d., 3 d)	- (-↓)	-	+	ND ¹	→	-	0/5	Deshmukh et al, 1982, 1983
ASA + FluB	-	-	+	ND	→	-	0/5	
ASA + FluB + Arg-diet	+	+	+	ND	↓	Encephalopathy Hyperammonemia	12/16	
Ferrets								
ASA (750 mg/kg, b.i.d.; 4 d)	ND	+	+	±	↓	Encephalopathy (-→coma)	6/13	Linnemann et al., 1979
FluB	ND	±	(+)	±	↓	Fever (mild)	0/11	
ASA + FluB	ND	±	+	±	↓	Encephalopathy (-→coma)	10/19	
Mice								
ASA	ND	-	-	?	→	-	-	Hug et al., 1981
? (500 mg/kg/d)								
EMC virus	ND	-	±	-	→	Encephalopathy	-	
ASA + EMC virus	ND	-	±	±	→	Encephalopathy	-	
Mice								
ASA (300-667 mg/kg)	ND	(+)	-	ND	ND	-	0/54	Davis et al., 1985,
FluB (concentrated)	+	+	+	ND	ND	Encephalopathy	32/60	Trauner et
ASA + FluB	ND	?	++	ND	ND	Encephalopathy	40/61	al, 1988

¹ND, not done or not reported

Fig. 1. Experimental protocol mouse model for Reye's Syndrome.

infected with sublethal doses of mouse-adapted human influenza B virus (FluB) (Crocker et al., 1986; Murphy et al., 1986,1987).

The chemical/viral mouse model for RS is set up as shown in Fig. 1. Male and female Swiss white mice (Charles River, St. Constant, Quebec) are mated at 12 wk of age in a ratio of 3 females to 1 male. The males are removed from the cages after 1 wk. Pups born on the day in which most births occur are used for the experiment, and this is considered Day 0 of the study. On Day 1, the age-matched pups are counted, pooled, and randomly distributed among the nursing mothers. Animals are housed in sets of 2 mothers with 2 litters (7–8 pups/litter) per cage. The neonates are divided into two groups, one of which is painted abdominally with Eagle's Minimal Essential Medium (MEM), and the other group are painted with a 1% solution (8.9 mg/100 mL MEM) of the industrial surfactant, Toximul MP8 (Tox) (Charles Tennant & Company, Toronto, Canada; Lot 9-30162). For the first 5 d, the mice receive 8.6 ± 2.6 mg Tox/d; this dosage is increased to 25.8 ± 9.0 mg/d for the last 6 d. In a limited number of our studies, Tox was administered in a single ip injection (Crocker et al., 1986).

The day after the painting is finished (i.e., Day 12), each of the two groups of mice are further subdivided, with half receiv-

ing virus under light ether anesthesia and the other half receiving ether alone. The four experimental groups are MEM, Tox, Virus (FluB), and Tox + FluB.

Mouse-adapted influenza B (Lee) virus was obtained originally from F. Ennis, National Institutes of Health, Bethesda, MD. The starting virus is passed seven times in CFW mice according to Ennis et al. (1976). Briefly, for each passage, five mice are inoculated intranasally under light ether anesthesia with 10 μ L of the starting virus. After 48 h, the mice are killed and lungs are removed, weighed, and homogenized with a tissue grinder in Hank's balanced salt solution (HBSS)(10% w/v) containing 2% (w/v) gelatin. After two freeze-thaw cycles, the homogenate is centrifuged at 800g for 10 min at 4°C to remove debris. The supernatant is diluted in HBSS, and 0.1 mL aliquots are inoculated into the allantoic cavities of 8-day-old fertilized hen eggs. After 48 h at 37°C, the allantoic fluid is collected, centrifuged (800g, 10 min), and stored in 0.5 mL aliquots at -80°C (virus stock). The LD₅₀ of the virus is determined by recording the mortality over a 7-day period after inoculating 10-12-d-old mice intranasally with different dilutions of the virus stock. For most experiments, the mice are inoculated with 10 μ L of a solution containing 640 HA U/0.5 mL.

In each experiment, a proportion (30-60/experimental group) of the total number of animals (approx 600) are set aside for mortality counts, which are carried out each day. The mortality rates vary from one experiment to another; however, mice given the combined Tox plus virus treatment consistently have a higher mortality rate than those given virus alone (Crocker et al., 1986; Murphy et al., 1986). The two aspects of this detergent + FluB model that best reflect the human disease are the selective ultrastructural damage to hepatic mitochondria (Crocker et al., 1986), and the increases in intracranial pressure (ICP) (Crocker et al., 1991). Neither effect is seen in animals exposed to detergent alone; virus infection alone does not affect the mitochondria, although it does produce moderate increases in ICP. The serum changes that are consistent with RS include hyperammonemia (also seen with detergent alone) and a decreased total lipid content (hypopanlipidemia). The livers of the combined-treat-

ment mice show no evidence of inflammation or necrosis, but have significantly reduced levels of ornithine transcarbamylase activity. The features that distinguish this model from the human disease are an apparent lack of increase in serum free fatty acids and an absence of hepatic fat. There are at least two possible explanations for these discrepancies, one of which is that they occur during the earlier periods of the experiment at times when no analyses were done, or (and perhaps more likely) the synergism between the detergent and virus with respect to fat abnormalities is a species-specific phenomenon. Support for the latter of these possibilities comes from observations that there is frequently hepatic fat accumulation when the infecting virus is the native mouse pathogen, encephalomyocarditis (EMC) virus. In the EMC model, which has been studied by our group (Crocker et al., 1974, 1976) and by others (Hug et al., 1981), the mice are exposed to either Toximul (our studies) or to Atlox 3409 F (ICI America Inc., Wilmington, DE) (Hug et al., 1981), and subsequently inoculated ip with the virus in amounts that are titered to produce a 30–50% mortality rate. Deaths generally occur between 2–4 d following inoculation. In addition to developing fatty liver, the mice treated with EMC and detergent have several other hepatic changes consistent with RS (e.g., mitochondrial structural abnormalities and enzymatic changes).

In addition to concerns that the detergent/FluB mouse model does not produce serum hyperfattyacidemia or fatty steatosis of the liver, there have been questions raised regarding the actual deposition of the detergents (or their catabolic byproducts) in the animal tissues, and regarding the cause of death. We have obtained preliminary data confirming the presence of 1,2-dibromoethane (a derivatized byproduct of Toximul) in mouse and human livers, and in human brain samples (Crocker et al., 1985) but we have not done a large number of analyses. An important question is whether death results from the lung infection or whether an encephalopathy is involved. The lungs of the mice are infected, and virus can be recovered from them; however, data concerning CNS involvement have been difficult to obtain from such a small animal. In recent studies in which we measured ICP using a scaled-down method

described for cats, we found that the mice treated with detergent plus FluB had significantly elevated values for ICP (Crocker et al., 1991). Our present focus is to determine whether there is a relationship between detergent exposure, lipid catabolism, and elevated ICP.

5. Summary

The fact that each of the models for RS that have been described to date fails to faithfully duplicate all of the known features of the human disease confirms our appreciation of its tremendous complexity. Three undisputed features of the human disease are:

1. The transient damage to hepatic mitochondria that is best exemplified by the ultrastructural changes;
2. The great increase in catabolism; and
3. The encephalopathy.

In most cases, these appear to be related to a viral illness, although several toxins have been identified that produce a RS-like illness. The overriding questions that remain unanswered are:

1. What is/are the factor(s) that either initiate the disease process or cause a host to respond in an abnormal manner to a viral infection;
2. What are the specific metabolic aberrations, and what is/are their interrelationships; and
3. In what sequence and with what time course do they occur?

These issues are certainly complicated by our knowledge that some toxins (both xenobiotic and metabolic) can produce at least some of the abnormalities that are seen in RS, but what is their precise relationship with this disease? In this regard, we are hampered by the fact that we often must derive cause-effect relationships on the basis of empirical measurements of substances whose levels can vary greatly both from one individual to another, and from one time period to another. Finally, in our attempts to mimic the disease in animals, we must appreciate that this may be a species-specific disease, one or more of its components may be species-specific, and/or individual events

within the disease process may differ from one species to another. In spite of these numerous considerations, we are slowly gaining valuable insight into the complex and perhaps subtle relationships between abnormal metabolism and a life-threatening encephalopathy.

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References

- Brown R. E. and Forman D. T. (1982) The biochemistry of Reye's Syndrome. *CRC Crit. Rev. Clin. Lab. Sci.* **17**, 247-296.
- Bourgeois C. H., Shank R. C., Grossman R. A., Johnsen D. O., Wooding W. L., and Chandavimol P. (1971) Acute aflatoxin B₁ toxicity in the macaque and its similarity to Reye's Syndrome. *Lab. Invest.* **24**, 206-216.
- Brownstein D. G., Johnson E. A., and Smith A. L. (1984) Spontaneous Reye's-like syndrome in BALB/cByJ mice. *Lab. Invest.* **51**, 386-395.
- Colon A. R., Ledesma B. S., Pardo V., and Sandberg D. H. (1974) Viral potentiation of chemical toxins in the experimental syndrome of hypoglycemia, encephalopathy, and visceral fatty degeneration. *Am J. Dig. Dis.* **19**, 1091-1101.
- Crocker J. F. S., Rozee K. W., Ozere R. L., Digout S. C., and Hutzinger O. (1974) Insecticide and viral interactions as a cause of fatty visceral changes and encephalopathy in the mouse. *Lancet* **2**, 22-24.
- Crocker J. F. S., Ozere R. L., Safe S. H., Digout S. C., Rozee K. R., and Hutzinger O. (1976) Lethal interaction of ubiquitous insecticide carriers with virus. *Science* **192**, 1351-1353.
- Crocker J. F. S. and Ozere R. L. (1979) The incidence and etiology of Reye's Syndrome in eastern Canada, in *Reye's Syndrome II*, (Crocker J. F. S., ed.), Grune and Stratton, NY, pp. 3-12.
- Crocker J. F. S. and Bagnell P. C. (1981) Reye's syndrome: Clinical review. *Can. Med. Assoc. J.* **124**, 375-382.
- Crocker J. F. S. (1982) Reye's Syndrome. *Semin. Liver Dis.* **2**, 340-352.
- Crocker J. F. S., Fung D., Hudson R., and Safe S. H. (1985) Examination of the role of surfactants in Reye's Syndrome, in *Reye's Syndrome IV* (Pollack J. D., ed.), The National Reye's Syndrome Foundation, OH, pp. 135-140.
- Crocker J. F. S., Renton K. W., Lee S. H., Rozee K. R., Digout S. C., and Malatjalian D. A. (1986) Biochemical and morphological characteris-

- tics of a mouse model of Reye's Syndrome induced by the interaction of influenza virus and a chemical emulsifier. *Lab. Invest.* **54**, 32-40.
- Crocker J. F. S. (1988) Role of exogenous agents in Reye's Syndrome in "Reye Syndrome" Royal Society of Medical Services. *Round Table Series*, No. 8, Oxford, England, pp. 45-52.
- Crocker J. F. S., Lee S. H. S., Love J. A., Malatjalian D. A., Renton K. W., Rozec K. R., and Murphy M. G. (1991) Surfactant-potentiated increases in intracranial pressure in a mouse model of Reye's Syndrome. *Exp. Neurol.* **111**, 95-97.
- Daugherty C. C., Gartside P. S., Heubi J. E., Saalfeld K., and Snyder J. (1987) A morphometric study of Reye's Syndrome. Correlation of reduced mitochondrial numbers and increased mitochondrial size with clinical manifestations. *Am. J. Pathol.* **129**, 313-326.
- Davis L. E., Cole L. L., Lockwood S. J., and Kornfeld M. (1983) Experimental influenza B virus toxicity in mice. A possible model for Reye's Syndrome. *Lab. Invest.* **48**, 140-147.
- Davis L. E., Green C. L., and Wallace J. M. (1985) Influenza B virus model of Reye's Syndrome in mice: The effect of aspirin. *Ann. Neurol.* **18**, 556-559.
- Davis L. E. (1986) Influenza B virus model of Reye's Syndrome in mice: Review of clinical, biochemical and pathologic studies. *J. Natl. Reye's Synd. Found.* **6**, 43-50.
- Davis L. E. and Kornfeld M. (1986) Mouse influenza B virus model of Reye's Syndrome. Encephalopathy and microvesicular fatty metamorphosis of the liver by influenza B virus following intravenous administration in mice. *Am. J. Physiol.* **122**, 190-192.
- Davis L. E. (1987) Influenza B virus model of Reye's Syndrome. Evidence for a nonpermissive infection of liver and brain. *Lab. Invest.* **56**, 32-36.
- DeLong G. R. and Glick T. H. (1982) Encephalopathy of Reye's Syndrome: A review of pathogenic hypotheses. *Pediatrics* **69**, 53-63.
- Deshmukh D. R., Maassab H. F., and Mason M. (1982) Interactions of aspirin and other potential etiologic factors in an animal model of Reye syndrome. *Proc. Natl. Acad. Sci.* **79**, 7557-7560.
- Deshmukh D. R., Deshmukh G. D., Shope T. C., and Radin N. S. (1983) Free fatty acids in an animal model of Reye's Syndrome. *Biochim. Biophys. Acta.* **753**, 153.
- Deshmukh D. R. and Thomas P. E. (1985) Arginine deficiency, hyperammonemia and Reye's Syndrome in ferrets. *Lab. Anim. Sci.* **35**, 242-245.
- Deshmukh D. R. (1985) Animal models of Reye's Syndrome. *Rev. Infect. Dis.* **7**, 31-40.
- De Vivo D. C. (1984) Do animals develop Reye Syndrome? *Lab. Invest.* **51**, 367-372.
- De Vivo D. C. (1986) Do animals develop Reye's Syndrome? *J. Natl. Reye's Synd. Found.* **6**, 17-37.
- Ennis F. A., Well M. A., Barry D. W., Daniel S., and Manechevitz J. (1976) Host defence mechanisms against influenza infection. II. Protection of

- mice with vaccines against A/Port Chalmers/1/73 and B. Hong Kong/S/72. *Post Grad. Med. J.* **52**, 338–344.
- Faraj B. A., Caplan D. B., Newman S. L., Ahmann P. A., Camp V. M., and Kutner M. (1984) Hypercatecholaminemia in Reye's Syndrome. *Pediatrics* **73**, 481–488.
- Frewen T. C. and Kisson N. (1987) Cerebral blood flow and brain oxygen extraction in Reye syndrome. *J. Pediatr.* **110**, 903–905.
- Gauthier M. D., Guay J., Lacroix J., and Lortie A. (1989) Reye's syndrome: A reappraisal of diagnosis in 48 presumptive cases. *Am. J. Dis. Child.* **143**, 1181–1185.
- Glasgow A. W. and Chase H. P. (1975) Production of the features of Reye's Syndrome in rats with 4-pentenoic acid. *Pediatr. Res.* **9**, 133–138.
- Greene C. L., Blitzer M., and Shapira E. (1988) Inborn errors of metabolism and Reye Syndrome: Differential diagnosis. *J. Pediatr.* **113**, 156–159.
- Hart M. A., Swisher J. A., and Caspers M. L. (1989) Alterations in plasma amino acids and hepatic enzymes in the 4-pentenoic acid model of Reye's syndrome. *Biochem. Pharmacol.* **38**, 1696–1698.
- Heubi J. E., Partin J. C., Partin J. S., and Schubert W. K. (1987) Reye's Syndrome: Current concepts. *Hepatology* **7**, 155–164.
- Hug G., Bosken J., Bove K., Linnemann C. C. Jr., and McAdams L. (1981) Reye's Syndrome simulacra in liver of mice after treatment with chemical agents and encephalomyocarditis virus. *Lab. Invest* **45**, 89–109.
- Hurwitz E. S. (1988) The changing epidemiology of Reye's syndrome in the United States: Further evidence for a public health success. *Am. Med. Assoc.* **260**, 3178–3180.
- Kang E. S., Gates R. E., and Wrenn E. L. Jr. (1982) Abnormal cellular regulation of lipolysis and phosphorylation in Reye's Syndrome. *Biochem. Med.* **27**, 180–194.
- Kang E. S., Olson G., Jabbour J. T., Solomon S. S., Heimberg M., Sabesin S., and Griffith J. F. (1984) Development of encephalopathic features similar to Reye Syndrome in rabbits. *Proc. Natl. Acad. Sci. USA* **81**, 6169–6173.
- Kilpatrick L. E., Polin R. A., Douglas S. D., and Corkey B. E. (1989) Hepatic metabolic alterations in rats treated with low-dose endotoxin and aspirin: An animal model of Reye's Syndrome. *Metabolism* **38**, 73–77.
- Koga Y., Yoshida I., Kimura M., Yamashita F., and Sinniah D. (1987) Inhibition of mitochondrial functions by margosa oil: Possible implications in the pathogenesis of Reye's Syndrome. *Pediatr. Res.* **22**, 184–187.
- La Montagne J. R. (1983) Summary of a workshop on disease mechanisms and prospects for prevention of Reye's Syndrome. *J. Infect. Dis.* **148**, 943–950.
- Linnemann C. C. Jr., Ueda K., Hug G., Schaeffer, A., Clark A., and Schiff G. M. (1979) Salicylate intoxication and influenza in ferrets. *Pediatr. Res.* **13**, 44–47.
- Mamunes P., DeVries C. H., Miller C. O., and David R. B. (1975) Fatty acid quantitation in Reye's Syndrome, in *Reye's Syndrome* (Pollack J. D., ed.), Grune and Stratton, New York, pp. 245–254.

- McWilliam R. C. and Stephenson J. B. P. (1985) Life-threatening intracranial hypertension in Reye's syndrome treated with intravenous thiopentone. *Eur. J. Pediatr.* **144**, 383-384.
- Mowat A. P. (1983) Reye's Syndrome: 20 years on. *Br. Med. J.* **286**, 1999-2001.
- Murphy M. G., Archambault-Schertzer L., Ackman R. G., and Crocker J. F. S. (1986) Serum lipid abnormalities in a chemical/viral mouse model for Reye's Syndrome. *Lipids* **21**, 378-382.
- Murphy M. G., Archambault-Schertzer L., VanKessel J., Digout S. C., Malatjalian D. A., and Crocker J. F. S. (1987) Hepatic lipid abnormalities in a chemical/viral mouse model for Reye's Syndrome. *Lipids* **22**, 217-223.
- Murphy M. G., Digout S. C., and Crocker J. F. S. (1989) Encephalopathy of Reye Syndrome. Studies of the pathogenetic process in animal models, in *Hepatic Encephalopathy*. (Butterworth R. F. and Pomier Layrargves G., eds.), Humana Press, Clifton, NJ, pp. 245-259.
- Nara T., Tsukiyama T., and Nishimoto H. (1988) The neuron-specific enolase as a marker of brain destruction in a case of Reye's Syndrome: Related to the treatment of brain edema. *Acta Paediatr. Scand.* **77**, 912,913.
- Ogburn P. L. Jr. (1976) Medium and long chain serum free fatty acids in a case of Reye's Syndrome. *South. Med. J.* **69**, 632,633.
- Ogburn P. L. Jr., Sharp H., Lloyd-Still J. D., Johnson S. B., and Holman R. T. (1982) Abnormal polyunsaturated fatty acid patterns of serum lipids in Reye's syndrome. *Proc. Natl. Acad. Sci. USA* **79**, 908-911.
- Partin J. S., McAdams A. J., Partin J. C., Schubert W. K., and McLaurin R. L. (1978) Brain ultrastructure in Reye's Syndrome. Acute injury and recovery processes in three children. *Neuropathol. Exp. Neurol.* **37**, 796-819.
- Pranzatelli M. R. and De Vivio D. C. (1987) Pharmacology of Reye Syndrome. *Clin. Neuropharmacol.* **10**, 96-125.
- Quint P. A. K. and Allman F. D. (1984) Differentiation of chronic salicylism from Reye Syndrome. *Pediatrics* **74**, 1117-1119.
- Rarey K. E., Davis J. A., and Deshmukh D. R. (1987) Response of epithelial cells of the choroid plexus in the ferret model for Reye's Syndrome. *Lab. Invest.* **56**, 249-255.
- Reye B. D. K., Morgan G., and Baral J. (1963) Encephalopathy and fatty degeneration of the viscera: A disease entity in childhood. *Lancet* **2**, 749.
- Robinson B. H., Taylor J., Cutz E., and Gall D. G. (1978) Reye's Syndrome: Preservation of mitochondrial enzymes in brain and muscle compared with the liver. *Pediatr. Res.* **12**, 1045-1047.
- Roe C. (1988) Metabolic disorders producing a Reye-like Syndrome in "Reye's Syndrome" (C. Wood, ed.) *Round Table Series No. 8*, Royal Society of Medical Services, Oxford, England, pp. 85-101.
- Rogan W. J., Yang G. C., and Kimbrough R. D. (1985) Aflatoxin and Reye's Syndrome: A study of livers from deceased cases. *Arch. Environ. Health* **40**, 91-95.
- Romshe C. A., Hilty M. D., McClung H. J., Kerzner B., and Reiner C. E.

- (1981) Amino acid pattern in Reye Syndrome: Comparison with clinically similar entities. *J. Pediatr.* **98**, 788–790.
- Rozee K. R., Lee S. H. S., Crocker J. F. S., and Safe S. (1978) Enhanced virus replication in mammalian cells exposed to commercial emulsifiers. *Appl. Environ. Microbiol.* **35**, 297–300.
- Schwarz K. B., Larroya S., Kohlman L., and Morrison A. (1987) Erythrocyte lipid abnormalities in Reye's Syndrome. *Pediatr. Res.* **21**, 352–356.
- Sinniah D., Baskaran G., and Leong K. L. (1981) Detoxification of margosa oil causing Reye's syndrome. *IRCS Med. Sci.* **9**, 369.
- Sinniah D. and Baskaran G. (1981) Margosa oil poisoning as a cause of Reye's syndrome. *Lancet* **1**, 487–489.
- Sinniah D., Baskaran G., Looi L. M., and Leong K. L. (1982) Reye-like syndrome due to margosa oil poisoning: Report of a case with postmortem findings. *Am. J. Gastroenterol.* **77**, 158–161.
- Sinniah D., Schwartz P. H., Mitchell R. A., and Arcinue E. L. (1985) Investigation of an animal model of a Reye-like syndrome caused by margosa oil. *Pediatr. Res.* **19**, 1346–1355.
- Sullivan-Boylai J. Z. and Corey L. (1981) Epidemiology of Reye's Syndrome. *Epidemiol. Rev.* **3**, 1–26.
- Tanaka K., Isselbacher K. J., and Sheh V. (1972) Isovaleric and alpha-methylbutyric acidemias induced by hypoglycin A: Mechanism of Jamaican vomiting sickness. *Science* **175**, 69–71.
- Tang T. T., Siegesmund K. A., Sedmak G. V., Casper J. T., Varma R. R., and McCreadie S. R. (1975) Reye Syndrome. A correlated electron-microscopic, viral, and biochemical observation. *Am. Med. Assoc.* **232**, 1339–1346.
- Taubman B., Hale D. E., and Kelley R. I. (1987) Familial Reye-like Syndrome: A presentation of medium-chain acyl-coenzyme A dehydrogenase deficiency. *Pediatrics* **79**, 382–385.
- Taylor D. A., Bagnell P. C., Renton K. W., and Crocker J. F. S. (1979) Intracellular changes induced by emulsifier-virus interaction in vivo and in vitro in the mouse, in *Reye's Syndrome II* (Crocker J. F. S., ed.), Grune and Stratton, New York, pp. 459–472.
- Thayer W. S. (1984) Inhibition of mitochondrial fatty acid oxidation in pentenoic acid-induced fatty liver. A possible model for Reye's Syndrome. *Biochem. Pharmacol.* **33**, 1187–1194.
- Thurlow P. M., Desau R. K., Newberne P. M., and Brown H. (1980) Aflatoxin B₁ acute effects on three hepatic urea cycle enzymes using semi-automated methods: A model for Reye's syndrome. *Toxicol. Appl. Pharmacol.* **53**, 293–298.
- Tonsgard J. H. (1989) Effect of Reye's Syndrome serum on the ultrastructure of isolated liver mitochondria. *Lab. Invest.* **60**, 568–573.
- Trauner D. A., Nyhan W. L., and Sweetman L. (1975) Short-chain organic acidemia and Reye's Syndrome. *Neurology* **25**, 296–298.

- Trauner D. A. and Huttenlocher P. R. (1978) Short-chain fatty acid induced central hyperventilation in rabbits. *Neurology* **28**, 940-944.
- Trauner D. A. and Adams H. (1981) Intracranial pressure elevations during octanoate infusion in rabbits: An experimental model of Reye's Syndrome. *Pediatr. Res.* **15**, 1097-1099.
- Trauner D. A. (1982) Pathologic changes in a rabbit model of Reye's Syndrome. *Pediatr. Res.* **16**, 950-953.
- Trauner D. A., Horvath E., and Davis L. E. (1988) Inhibition of fatty acid beta oxidation by influenza B virus and salicylic acid in mice: Implications for Reye's syndrome. *Neurology* **38**, 239-241.
- Venes J. L., Shaywitz B. A., and Spencer D. D. (1978) Management of severe cerebral edema in the metabolic encephalopathy of Reye Johnson syndrome. *J. Neurosurg.* **48**, 903-915.
- Yoder M. C., Egler J. M., Yudkoff M., Chatten J., Douglas S. D., and Polin R. A. (1985) Metabolic and mitochondrial changes that mimic Reye Syndrome after endotoxin administration to rats. *Infect. Immun.* **47**, 329-331.