In vitro denaturation and (or) alteration of protein function by detergents have been extensively documented. I suggest that similar biochemical and clinical features of Reye's syndrome, sudden infant death syndrome, acute pancreatitis, and diabetic ketoacidosis may be explained as sequelae of the toxic detergent effects of nonesterified fatty acids and lysolecithins. These diseases may be provoked by a drug-induced diminution of the detergent-buffering capacity of blood or tissue proteins; by excess detergents produced in vivo, consequent to lipase activity induced by viral infection or metabolic disease; or by some combination of these factors.

The biochemistry and metabolism of fatty acids have been extensively studied by biochemists for years. Many review articles emphasize the role of fatty acids in nutrition. A voluminous literature is available on the possible role of lipids in heart disease. Nevertheless, whereas many large clinical laboratories measure triglycerides, cholesterol, and lipoproteins, and do lipoprotein electrophoresis, few measure nonesterified fatty acids (NEFA) or lysolecithins, and those that do receive relatively few requests. Even fewer laboratories offer quantification of specific nonesterified fatty acids.

In this article, I first review the detergent properties of these unique classes of lipids and their interactions with proteins. Then I present evidence that these molecules may be extremely important in certain diseases and that their measurement may assist in the understanding and control of these diseases.

The aspect of NEFA that give them special properties is their dual polar–nonpolar nature. A NEFA anion has a polar (carboxylate) end and a non-polar (alkyl) end; the polar end is water-soluble; the non-polar end is oil-soluble. Oil droplets in water coalesce so that there is an oil layer and a water layer, but in the presence of NEFA anions (soap) this changes so that the non-polar ends of soap molecules dissolve in the oil droplet, leaving the carboxylate ends projecting into the water layer. Each oil droplet is thus surrounded by a negatively charged atmosphere. Their mutual repulsion keeps the oil droplets from coalescing, and the result is a stable emulsion of oil in water. This emulsifying property of NEFA is shared with other molecules, such as lysolecithins, that contain a large non-polar portion and a polar portion. Such molecules are by definition detergents.

Lecithins, more precisely termed phosphatidylycholines, contain two esterified fatty acids, usually one saturated and the other unsaturated (1). Lysolecithins are produced by the action of phospholipase A₂ (EC 3.1.1.4) on lecithins, which removes one of the fatty acids (usually the unsaturated fatty acid) from lecithins, leaving a 1-acylglycerophosphocholine. Removal of this fatty acid residue from lecithins makes the resulting lysolecithins more polar, and they become strong detergents capable of impairing the structure and function of biological membranes (2–5), of direct lytic effects on neurons, and of inducing demyelination in peripheral nerves (2). Lysolecithins cause hemolysis or increased fragility of erythrocytes (5), and they can induce cardiac arrhythmias in experimental animals in concentrations lower than those found in serum (4). Serum proteins reverse the cardiotoxic effect of lysolecithins (4). The lysolecithins produced by the action of phospholipase A₂ are toxic; indeed, lysolecithins are the predominant toxic material produced by the phospholipase that is the active component of cobra and rattlesnake venoms (6).

Sodium lauryl sulfate, the most commonly used commercial detergent, is an analog of an NEFA, lauric acid, in which the sulfonic acid salt -OSO₂Na+ replaces the carboxylate anion. This same detergent has also been widely used as a protein denaturant, and NEFA themselves have also been implicated as protein denaturants (7–12).

What is the mechanism of this denaturation? There is good evidence that formation of apolar bonds is one of the most important, if not the most important, of the factors influencing the tertiary structures of proteins (13). The driving force for the formation of these bonds appears to be primarily entropic. The positive change in entropy that results from the liberation of water molecules organized about the apolar side chains of certain amino acid residues in the protein that accompanies the transfer of these side chains from an aqueous to a nonaqueous environment (the interior region of the protein molecule) is the major thermodynamic force for formation of apolar bonds (13). Detergents denature proteins because their hydrophobic ends react with hydrophobic side chains of proteins to form hydrophobic bonds, which are primarily responsible for tertiary protein structure and thus for maintaining the necessary conformation of those biologically active sites responsible for binding phenomena or enzymatic activity. Therefore, disruption of hydrophobic bonds by detergents leads to a corresponding loss of the biochemical activity of a protein. Denaturation of proteins by detergents has been demonstrated for so many different proteins that we can conclude that it is a general

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phenomenon (14). Table 1 lists such studies reporting that detergent denaturation of protein function occurs in various species and in proteins from multiple organs, and that it affects both enzymatic and binding activities.

Concentrations of NEFA sufficient to alter biochemical function are found in many disease states (15). For example, concentrations in serum as high as 3.0, 3.8, and 1.1 mmol/L have been found in bacterial infection, diabetes mellitus, and hyperthyroidism, respectively. The rate of denaturation has been shown to vary with different proteins, but all proteins lose tertiary structure and biological activity at a high enough detergent concentration (14). Moreover, the potency of denaturation of proteins by NEFA appears to depend on the particular type of NEFA. Nonesterified fatty acids in serum have an even number of carbons up to 20 and may have from zero to as many as five double bonds. Shaw et al. (11) found that the degree of denaturation of thyroxin-binding globulin increases with decreasing chain length for both saturated and unsaturated NEFA but increases even more with the number of double bonds. Almost identical results were obtained for denaturation of alpha-fetoprotein by NEFA (9), for alteration of mitochondrial function by NEFA (16), and for denaturation of digoxin antibodies (12).

In each case, NEFA in concentrations as low as 0.1 mmol/L caused some denaturation or alteration of protein or mitochondrial function.

In serum the predominant NEFA are stearic, palmitic, oleic, lauric, myristic, myristoleic, palmitoleic, linoleic, and arachidonic acids—oleic acid being the major unsaturated NEFA and palmitic acid the major saturated NEFA (17).

Most significantly, both the total NEFA concentration and the concentration of these individual fatty acids change substantially with age. Caffeine, cigarette smoking, diet, and duration of fasting all influence NEFA concentrations, so control of these variables is essential for a study to be valid. In a study in which these variables were controlled (18), it was found that the values for the absolute concentration of each of the above fatty acids and for total NEFA decreased exponentially with age over the age range evaluated (eight to 25 years). For newborns, the upper limit of the normal reference interval was more than twice that for adults (18).

Classically, albumin has been considered the most important carrier of NEFA in plasma, but in light of extensive evidence that detergents bind to all proteins, it appears that the importance of albumin in NEFA binding may be related more to the fact that albumin is the most predominant protein in serum rather than to any peculiar NEFA transport ability. Detergent binding to proteins appears to be a general property of all proteins, but the NEFA—albumin system has been most extensively studied while other systems have been somewhat neglected, although not completely (19–23).

Regardless of the issue of the specificity of albumin transport of NEFA, the high concentration of albumin in serum makes it quantitatively the most important protein for the modulation of NEFA effects; in comparison, this effect of all other proteins in serum is small. Nevertheless, an increase in NEFA would lead to a corresponding tendency toward denaturation of other proteins. NEFA concentrations are much higher in the serum of children, but the albumin concentration is the same in adults and in children. Thus the detergent-buffering capacity of serum is inversely related to age up to adulthood.

The biochemistry and pathology of several disease states are consistent with such an in vivo NEFA buffering system and with the notion that, when the detergent-buffering capacity of the protein is exceeded, the resulting free NEFA may damage many different tissues by their detergent action. I now examine the evidence that this is the case.

Reye's Syndrome

Reye's syndrome usually follows a viral illness (most commonly chickenpox and influenza) and is associated with nausea, headache, lethargy, coma, and death (24). Increased intracranial pressure, seizures, and abnormal electroencephalograms are frequently noted. Ingestion of salicylates is associated with increased risk of developing Reye's syndrome (25). The activities of the aminotransferases in serum are at least threefold the upper normal limits, prothrombin time is prolonged, and the blood ammonia concentration is usually increased (25). Severe abnormalities in platelet counts or coagulation factors (or both) and, occasionally, disseminated intravascular coagulation have been noted (26). There is a definite association with acute pancreatitis (27, 28), the significance of which will be discussed later in detail.

Serial measurements of total NEFA in serum show an increase during Reye's syndrome and a decline during convalescence and after recovery (24). The mean NEFA value in one series of Reye's syndrome patients (29) was 1.21 mmol/L, as compared with 0.29 mmol/L in a group of age-matched controls, a statistically significant difference (p <0.0005). Measurement of serum NEFA also has important prognostic value. Pollack et al. (29) found that only three of nine patients with NEFA values >0.85 mmol/L survived, while 10 of 13 with values <0.85 mmol/L survived (29). In Reye's syndrome the NEFA that are most significantly increased as compared with controls are the short- and medium-chain (C6 to C12) saturated fatty acids (30) and the long-chain (C16 to C20) unsaturated fatty acids (24). Values for some of the mean polyunsaturated fatty acids in sera from Reye's syndrome patients were 25-fold normal (24), and one such patient had an octanoic acid concentration in serum that was 500-fold the highest control value and a lauric acid concentration 50-fold the mean control value (30).

Those NEFA that are most significantly increased in Reye's syndrome are the same as those that are the most powerful protein denaturants and inhibitors of mitochondrial function. Sera from patients with Reye's syndrome had normal values for triglyceride, total cholesterol, free cholesterol, and lipid phosphorus but abnormally low total lipids (29). The fact that for the decrease in the proportion of each of the polyunsaturated fatty acids in the phospholipids there is a corresponding increase in the polyunsaturated fatty acid content of the NEFA fraction suggests the formation of lysolecithins, mediated by phospholipase A2 (24).

Therapies that have been found effective in Reye's sy-
drome are consistent with the hypothesis that NEFA is the toxic agent and that decreasing NEFA should diminish the severity of symptoms.

Intravenous infusion of glucose and insulin, documented as effective therapy (25), is associated with a marked decrease in NEFA (24). For example, a patient with a serum NEFA concentration of 12.2 mmol/L, so treated, showed a decrease to 0.375 mmol/L (30). Insulin decreases NEFA by inhibiting lipolysis.

Barbiturates are commonly used to treat the neurological symptoms of Reye's syndrome (25). Shiu and Nemoto (31) have shown in experimental animals that decreased NEFA liberation may be the biochemical basis for barbiturate attenuation of ischemic brain injury.

Exchange transfusions are also commonly used to treat Reye's syndrome (24, 25). NEFA concentrations in plasma of a patient who recovered from this syndrome were markedly lower after transfusion (24), consistent with the hypothesis that Reye's syndrome is caused by toxic NEFA concentrations and that decreasing them—in this case by exchange transfusion—alleviates the symptoms of the syndrome.

Results of in vitro studies and of the experimental animal model of Reye's syndrome seem to support the hypothesis that excess NEFA are responsible for the biochemical and clinical findings in Reye's syndrome. Mitochondrial swelling is a consistent finding in liver biopsies from patients with Reye's syndrome (32). Similar swelling has been induced by NEFA in liver mitochondria in vitro at a concentration of 13 μmol/L (16). Infusion of NEFA caused tissue damage in many other organs (33). Indeed, mitochondria from the liver, skeletal muscle, and pancreas of patients with Reye's syndrome all exhibit similar histological patterns of injury (32), implicating a generalized toxic agent in Reye's syndrome. The apparently complete return to normal of many of the survivors of Reye's syndrome is consistent with the reversibility of detergent–protein interactions, i.e., the reversibility of changes in tertiary structure that have not gone too far. Alterations in biochemical function caused by detergent-denaturation of proteins can readily be restored by removing the detergent (13).

The best evidence that the presence of NEFA in excess of the capacity of serum to bind them can account for the altered mitochondrial function observed in Reye's syndrome is provided by Ansevin (34). Addition of serum from patients with Reye's syndrome to mitochondria isolated from rat brain stimulated their ATPase activity and restored respiratory rate and decreased their respiratory control (modulation of respiratory rate by the ratio of adenosine diphosphate and triphosphate) and rate of phosphorylation. Sera from normal individuals did not have these effects, nor did sera collected during convalescence from survivors of Reye's syndrome. The severity of this derangement of mitochondrial function was greater in sera from patients who were deeply comatose, and it also correlated with higher values for NEFA in serum from these patients. Moreover, dialysates of NEFA-containing serum from a patient with Reye's syndrome had the same effect on mitochondrial function as did whole serum, and increased amounts of dialysate completely blocked phosphorylation. Preincubation of patients' sera with albumin returned values for mitochondrial function to normal. And, finally, when NEFA were added to control sera at the same concentrations found in Reye's syndrome and then incubated with mitochondria, mitochondrial functions were altered to the same extent as by Reye's syndrome sera.

A good experimental animal model of Reye's syndrome is produced by infusing NEFA. Rabbits so treated develop encephalopathy, increased intracranial pressure, electroen-

Several cases of carnitine deficiency have been described (17). Carnitine deficiency shares symptoms with Reye's syndrome: hepatomegaly, hyperammonemia, increased serum transaminases, and coma occurring after mild respiratory tract infection. Because carnitine is needed to transport fatty acids across the inner mitochondrial membrane, a deficiency of carnitine can lead to a marked increase in NEFA—especially during fasting, when adipose tissue converts large quantities of triglycerides to NEFA. In one case of carnitine deficiency (36), NEFA increased by more than 20-fold during a 32-h fast. This increase in NEFA was associated with sudden cardiorespiratory arrest characterized by an absence of cardiac electrical activity.

A syndrome similar to Reye's syndrome, described by workers in India, occurs after margosa oil is ingested. This oil, from the Indian nim tree, consists mainly of glycerides (esters of NEFA and glycerol) that are converted to NEFA in the small intestine. This oil is used in India as salicylates are in the United States: to treat fever, respiratory infection, and arthritis. A syndrome of vomiting, drowsiness, metabolic acidosis, and encephalopathy has been reported in infants within hours of ingestion of this oil (37). Its administration to mice results in mitochondrial changes, fatty infiltration of liver, and cerebral edema, similar to the changes described in Reye's syndrome (37). Pathological changes similar to Reye's syndrome on autopsy have been reported in an infant who died from margosa oil poisoning with a Reye-like syndrome (37). As in Reye's syndrome, an upper respiratory infection may also be associated with the toxic response to margosa oil. Absorbed NEFA is re-esterified into glycerides in the intestinal mucosa when an adequate supply of free coenzyme A is available for activation of NEFA. When free coenzyme A is depleted, owing to anorexia, NEFA may enter the portal system directly instead of being converted to triglycerides in chylomicrons.

Several inborn errors of metabolism, including maple syrup urine disease, propionic acidemia, isovaleric acidemia, and beta-hydroxy-beta-methylglutaric aciduria, commonly present with the clinical features of Reye's syndrome (38). All of these diseases are associated with the excretion of large quantities of nonesterified carboxylic acids.

Reye's syndrome has also been reported as a toxic side effect of valproic acid administration (39). Valproic acid is itself a short-chain nonesterified fatty acid used as an antiepileptic drug. It also competes with other NEFA for binding sites to serum albumin, thus diminishing detergent-binding capacity of serum as salicylates do (15). Thus, Reye's syndrome associated with valproic acid appears to be due to generalized protein denaturation due to decreasing the detergent-binding capacity of serum, or to a direct protein denaturation by valproic acid itself, or to both.

Many other drugs also compete with NEFA for albumin binding sites and thus diminish the detergent-binding capacity of albumin (40). Salicylate was found (40) to be one of the more commonly used drugs that competes strongly with NEFA for binding to albumin. Salicylate is so commonly used that this effect is of especial significance when the concentrations of NEFA and other detergents are increased. An exact value for this binding capacity cannot be given, because different NEFA, lyssolecithins, and drugs react somewhat differently with albumin, but the value of 1.7
mmol/L, at a normal protein concentration, as determined in the experiments of Rudman et al. (40) would be a good starting approximation. Because the therapeutic limit for salicylate (up to 200 mg/L) can also be expressed as 1.44 mmol/L, it can be readily appreciated that the detergent-binding capacity will be on the verge of saturation, even in healthy adults, for whom the NEFA range is 0.2 to 0.5 mmol/L (15). Children have higher NEFA concentrations than adults, so even a lower salicylate concentration will saturate the detergent-binding capacity of their serum, allowing the resulting unbound NEFA to enter the cells. Thus, it is not surprising that the symptoms of salicylate toxicity are similar to those of Reye's syndrome (25).

As we have seen, an excess of NEFA is associated with a set of clinical symptoms (a syndrome) in diseases with widely different biochemical precipitating events. The biochemical feature common to these conditions appears to be an excess quantity of nonesterified fatty acids and (or) other detergents such as lysolecithins. Children, as mentioned, have higher NEFA values than adults (15), thus predisposing them to toxic effects of detergents produced by lipolysis.

The increased NEFA in carnitine deficiency, margosa oil poisoning, other certain inborn errors of metabolism, and in salicylate and valproic acid toxicity can be readily explained. But what explains the NEFA increase associated with Reye's syndrome after viral infection? Several factors are probably important.

First, viral infection often causes liver dysfunction (24). Because the liver is a major site of NEFA catabolism, hepatic dysfunction provoked by infection can lead to increased serum NEFA (24). Second, the decrease in phospholipids and increase in unsaturated NEFA in Reye's syndrome is consistent with increased phospholipase activity (24) as increased lysolecithin. Viral infection is associated with increased phospholipase activity (24). Reye's syndrome was seen in an adult infected with dengue virus (41), which increases phospholipase during cellular invasion (42).

Lipase is also commonly released as a result of pancreatitis after viral infections in children (43). Acute pancreatitis is commonly associated with Reye's syndrome (27, 28), and many of the same symptoms are common to both diseases. The pulmonary alveolar cells are also rich in lipase (44), and upper respiratory infections are probably the cause of lipase release in Reye's syndrome after upper respiratory infection.

Another consideration is that viral infection often causes loss of appetite and (or) vomiting, which leads to decreased secretion of insulin and increased release of NEFA owing to lack of insulin inhibition of lipolysis. Reye's syndrome is also associated with a marked increase in the lipolytic stress hormones (34), and the syndrome usually occurs during or after recovery from a viral infection when the appetite is returning to normal. A sudden influx of NEFA from digested dietary fat without sufficient cofactors for re-esterification in the intestinal mucosal cells could represent the critical additional concentration of NEFA that exceeds the detergent-binding capacity of serum and starts a vicious, and often fatal, circle: NEFA are hepatotoxic (24) and when their concentration exceeds the binding capacity of serum, the ability of the liver to metabolize NEFA would be impaired, leading to further increases in NEFA and further hepatotoxicity.

Incubation of NEFA with liver homogenates in vitro decreased the activities of carbamoylphosphate synthase (ammonia) (EC 6.3.4.16) and ornithine transcarbamylase (EC 2.1.3.3) (45). Inhibition of these urea-cycle enzymes by NEFA may explain the hyperammonemia and the decreased activity of these urea-cycle enzymes in livers from patients with active Reye's syndrome.

Sudden Infant Death Syndrome

Sudden infant death syndrome (SIDS) is the most frequent cause of death in infants. About one baby in 500 dies unexpectedly between one week and two years. This syndrome often follows mild respiratory infection. Lethargy, coma, and apneic death during sleep rapidly follow the onset of symptoms. The similarity of this disease to Reye's syndrome has been recognized, and the difficulty in differentiating the pathology of the two disorders has been noted (46).

The findings of Sinclair-Smith are particularly dramatic (47). Thirty-five percent of autopsy cases showed gross fatty changes in the liver, and fatty involvement of the kidneys and heart were reported (46). In another large series of SIDS victims, over 90% of the subjects showed fatty change of the liver, which in 5% was severe (47). Fatty changes in the area of the tapetum were found in half of 41 SIDS victims (47). There is markedly decreased phospholipid and markedly increased lysolecithin in lung surfactant from SIDS cases (48); such changes in lung surfactant in SIDS suggest increased phospholipase.

Treatment with phenothiazine has been implicated as a risk factor for SIDS. Phenothiazine-containing medication is commonly used in Belgium and other European countries for the treatment of nasopharyngitis. In a prospective study by Kahn and Blum (49), SIDS victims, "near-miss" cases, and controls were compared for the coexistence of nasopharyngitis and phenothiazine treatment preceding death or hospitalization. The incidence of nasopharyngitis was comparable in the three groups, but phenothiazines were used 11 times more frequently in the fatal and near-miss cases. Phenothiazines have detergent properties like lysolecithin, including a well-known ability to lyse erythrocytes (50). Phenothiazines also bind to albumin like salicylates and thus decrease the detergent-binding capacity of albumin (50).

Perhaps the most important findings in SIDS were the detection of a low concentration of insulin in the serum (51) and of ketone bodies in the vitreous humor (52), consistent with NEFA overload as in diabetic coma. Because the value for glucose is normal or low in SIDS victims (53), these infants do not appear to have classic diabetes mellitus.

In addition, the mean NEFA concentration in plasma from 12 SIDS victims (0.93 ± 0.24 mmol/L, mean ± SD) measured by an enzymatic assay (54) in this laboratory (unpublished data) is very similar to the previously mentioned cutoff NEFA concentration in the sera of Reye's syndrome victims who do not survive (0.85 mmol/L). 1

Acute Pancreatitis

Acute pancreatitis has a prevalence of 0.5%. Abdominal pain, nausea, vomiting, and abdominal distention are frequent complaints. Activation by viruses of proenzymes within the pancreas instead of, as normally, in the intestine, or trauma or other agents are believed to cause this disorder. Complications of this disease include impairment of myocardial contractility with ST-segment and T-wave abnormalities appearing in the electrocardiogram that simulate myocardial ischemia; disseminated intravascular coagulation; pulmonary complications, including respiratory distress syndrome; central nervous system symptoms,

1 Plasma samples from SIDS victims were obtained from Dr. Tyson Tildon of the SIDS Institute, Department of Pediatrics, University of Maryland School of Medicine, Baltimore, MD. These samples were obtained during postmortem examinations from infants who had died suddenly or unexpectedly and whose autopsies failed to reveal an adequate explanation. After centrifugation, all plasma was kept frozen at −70 °C until shipped to this laboratory on solid CO₂.
including psychosis and encephalopathy; and sudden death (55). The diagnosis of acute pancreatitis is established by increased activity of amylase and (or) lipase in the serum. Hypo-albuminemia, if present, is associated with more-severe disease and increased mortality. About 25% of such patients have hypoxemia, which may precede or accompany the adult respiratory distress syndrome. Hyperglycemia is common, as a result of decreased insulin release, increased glucagon release, and increased release of glucocorticoids and catecholamines. Release of phospholipase and lipase into the bloodstream and surrounding tissues causes a marked increase in the detergents, the NEFA, and lysolecithins. The increased mortality associated with hypo-albuminemia is readily explained on the basis of the present hypothesis. The patient with acute pancreatitis and hypo-albuminemia has a sudden increase in NEFA and lysolecithins, but the capacity of his albumin to buffer these detergents is diminished.

Schmitz-Moorman and Boger (33) demonstrated that producing NEFA by directly injecting olive oil and lipase into liver, kidney, pancreas, skeletal muscle, and perivascular sheath caused massive necrosis within 3 h, with subsequent spread, and that injection of oleic acid had similar effects. In another study (56), the parenchymatous lesions in acute pancreatitis in rats were shown to be due to the detrimental action of released NEFA and, based on comparative pathological studies of tissue obtained at autopsy from humans who died of acute pancreatitis, it was concluded that acute pancreatitis in humans is initiated by lipase, which releases cytoxic NEFA. Short-term vascular changes ascribable to the detergent action of NEFA included transendothelial emigration of leukocytes, vacuolization of endothelial cells, subendothelial edema, fragmentation of the basement membrane, and subendothelial deposits of plasma proteins. When NEFA acted for 24 h, massive vascular necrosis occurred.

Diabetes Mellitus and Ketoacidosis

One of the important actions of insulin is to decrease serum NEFA concentrations by inhibiting lipolysis of adipose tissue (57). Many studies document the increased NEFA concentrations in juvenile as well as maturity-onset diabetes mellitus (57, 58). The incidence of atherosclerosis, stroke, and coronary heart disease is increased in diabetes (58). Peripheral vascular insufficiency manifested as ischemia, ulceration, or gangrene of the limbs also is commonly associated with diabetes (58).

Reinila (59) showed that abnormally high NEFA concentrations in the blood are associated with morphological changes in the arteries of diabetic rats, and that the severity of the lesions increased in parallel with an increase in the NEFA/albumin ratio, which is consistent with the role of albumin, as the major serum protein, in protecting the organism from the toxic detergent action of NEFA. Geesel et al. (60) found increased venous platelet aggregates in fasting individuals with high NEFA concentrations, and it was later demonstrated (61) that albumin protects against this aggregation while NEFA accelerates platelet aggregation. These studies and those done on the effect of NEFA on diabetes, on platelet function, and on the vascular system in pancreatitis indicate a toxic detergent effect of NEFA on the cardiovascular system when NEFA concentrations exceed the detergent buffering capacity of serum and well may indicate an important role of NEFA and other detergents on the development of atherosclerosis in non-diabetics.

Symptoms of diabetic ketoacidosis include hyperglycemia, ketosis, and acidosis, all due to acute insulin deficiency. This disorder is also associated with increased NEFA. Some of the clinical symptoms of diabetic ketoacidosis are the same as those of acute Reye's syndrome: nausea, vomiting, tachypnea, stupor or coma, and cerebral edema with increased intracranial pressure.

What Feature Do the Above Disorders Share?

Table 2 summarizes the clinical and laboratory findings in the preceding disorders. NEFA concentrations are increased in all four disorders and increased lysolecithins have been documented in all except diabetes mellitus, for which no information was found. Ketosis has been reported in ketoacidosis of diabetes mellitus, Reye's syndrome, and sudden infant death syndrome. Blood glucose is at normal upper limits in ketoacidosis and is frequently above normal in acute pancreatitis but low or normal in Reye's syndrome and sudden infant death syndrome.

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Prolonged prothrombin time is a common finding in Reye's syndrome (25, 26) and in acute pancreatitis (55). Coagulation disorders, including disseminated intravascular coagulation, occur as complications of diabetes (60, 61), acute pancreatitis (55), and Reye's syndrome (26). Blood removed from the hearts of mice victims is almost always liquid (53), which indicates that there is also a coagulation disorder in this disease. The prolonged prothrombin time in Reye's syndrome and acute pancreatitis and the presence of disseminated intravascular coagulation appear to be causally related. If platelets are diminished because of widespread intravascular coagulation, the prothrombin time becomes abnormal because other coagulation factors are also consumed. The products of inappropriate fibrinolysis actively inhibit clot formation and additionally prolong the clotting time in many cases of disseminated intravascular coagulation. Increased platelet aggregation can be induced by increased NEFA (60) and can be prevented by albumin (61).

NEFA have been implicated as the cause of respiratory failure in acute pancreatitis (62), and injection of NEFA into rabbits was followed by disruption of the pulmonary capillary endothelium and pulmonary edema (62). Injection of NEFA into the pulmonary artery of dogs caused acute respiratory failure (62). Infusion of a NEFA, oleic acid, into an isolated pulmonary lobe significantly decreased its compliance. When albumin was added immediately after this NEFA infusion, lung compliance was normal (62). Respiratory distress is a feature common to all four of the diseases in Table 2. The pathological features in the lung due to sites (53), adult respiratory distress syndrome of acute pancreatitis (63), experimental respiratory distress syndrome induced by NEFA (62), and Reye's syndrome (63) are the same: hyperemia, edema, and intra-alveolar hemorrhage.

Nausea and vomiting are premonitory symptoms in pancreatitis (55), Reye's syndrome (25, 32), and diabetic ketoacidosis (57). Coma and abnormal mental behavior have been reported in all of the four diseases in Table 2 (25, 55, 57, 64). Encephalopathy occurs in Reye's syndrome (34), is a complication of pancreatitis (55), and in one study neuropathological changes were detected in infants dying of sites, including fatty changes in the tapetum of the brain in over half the infants (53). Neurupathy of the brain, spinal cord, and peripheral nerves appears to be an integral part of the diabetic syndrome (57). Cardiac changes have been reported in sites (51, 53), acute pancreatitis (55), and Reye's syndrome (36), and cardiovascular disease is widely recognized as a common complication of diabetes mellitus (57). These cardiac effects are consistent with the toxic effects of NEFA (65–68) and lysolecithin (4) on the heart and with the reported oxygen-wasting effects of detergents on cellular respiration.

The increased incidence of sites (53) and Reye's syndrome (25, 32) during influenza A, influenza B, and varicella outbreaks and the common occurrence of these diseases and diabetic ketoacidosis after viral infection (57) are consistent with the increased NEFA and lysolecithins produced by possible viral-induced phospholipase A2. The increased incidence of acute pancreatitis, a rare disease in children, in Reye's syndrome (27, 28) is consistent with the hypothesis that any condition that causes excess detergents to be produced will result in a clinical syndrome indistinguishable from Reye's syndrome.

Several important questions remain to be answered.

First, NEFA also are increased in serum in fasting and starvation. Why does Reye's syndrome not occur in these states? The source of the increase in Reye's syndrome apparently is phospholipids, while triglycerides from adipose tissue are the source of NEFA during fasting and starvation. No disproportionate increases in short-chain saturated or long-chain unsaturated fatty acids or lysolecithins have been reported in fasting or starvation. Thus, the NEFA mobilized during fasting and starvation are not those with the protein-denaturation potential of those that appear in the serum in Reye's syndrome.

Secondly, why do some patients with Reye's syndrome with supranormal serum NEFA concentrations survive while other patients with whose serum NEFA is <0.85 mmol/L do not? The concentrations of lysolecithins need to be correlated with the NEFA concentrations, because both probably contribute to the toxicity. Different NEFA differ widely in their denaturation effects, so even the measurement of the total concentrations of both NEFA and lysolecithins may only crudely approximate the toxic potential of these compounds. Clearly, biochemical assays or bioassays (or both) are needed that measure the toxic potential of these substances.

Much more work needs to be done on understanding the dynamics of transfer of NEFA and lysolecithins to the tissues where biochemical damage occurs and to assess the detergent-buffering system of the tissues where the pathological changes occur. Other possible toxic effects of NEFA due to increased prostaglandin production should also be explored (24).

References
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