Liver Function in the Malnourished Child

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At the time when "nutritional cirrhosis" was a viable concept, there was great enthusiasm for research to confirm the proposed sequence: Malnutrition → Fatty liver → Cirrhosis. It became clear that it was difficult to mimic the first of these steps in the laboratory, and the second could not be achieved without resorting to choline deficiency, so enthusiasm dwindled (1,2). Now, however, the situation has changed. Whether aflatoxin (3) and/or free radicals (4) have anything to do with kwashiorkor (Fig. 1), the suggestion that they may, coming at a time when molecular genetics is sweeping into clinical nutrition research, brings a fresh impetus to the study of the liver in malnutrition. The relationship between malnutrition and liver function is multifaceted and requires broad interpretation of both terms. Thus, "malnutrition" must include overall protein-energy deficiency, specific deficiency of dietary components, overall energy excess, specific excess of dietary components, and abnormal route of nutrition (parenteral nutrition) (Table 1). Liver "dis-
TABLE 1. Etiology of total parenteral nutrition (TPN) cholestasis in preterm infants (5,6)

<table>
<thead>
<tr>
<th>Etiology</th>
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<tbody>
<tr>
<td>Physiological cholestasis (7)</td>
</tr>
<tr>
<td>Fasting</td>
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<tr>
<td>Serum levels of secretin, glucagon, gastrin, and motilin are low at birth and rapidly increase with feeding. This response is blunted in preterm infants. Glucagon stimulates hepatic bile acid uptake.</td>
</tr>
<tr>
<td>Bile acids</td>
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<tr>
<td>Ileal disease or resection increases bile acid load entering colon. Colonic transport is delayed. There is conversion by colonic bacteria of chenodeoxycholate (nontoxic) to lithocholate (hepatotoxic), and of sulfated lithocholate (nonreabsorbable) to lithocholate (reabsorbable). In adults, biliary lithocholate is increased with TPN, and liver damage is possibly preventable with metronidazole.</td>
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<tr>
<td>Bacteria</td>
</tr>
<tr>
<td>Neonatal sepsis without TPN → cholestasis.</td>
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<tr>
<td>Endotoxin inhibits bile flow.</td>
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<tr>
<td>Amino acids in infusate</td>
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<tr>
<td>Amino acids depress bile acid uptake by hepatocytes.</td>
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<tr>
<td>Methionine in high concentration obliterated bile salt–independent bile flow in isolated hepatocytes.</td>
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<tr>
<td>Photodegradation products of tryptophan were hepatotoxic in animal experiments.</td>
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<tr>
<td>Taurine deficiency impairs bile acid conjugation.</td>
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<tr>
<td>Extrahepatic obstruction</td>
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<tr>
<td>Biliary sludge, gallstones.</td>
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</table>

TABLE 2. Examples of hepatotoxic xenobiotics (8)

<table>
<thead>
<tr>
<th>Type</th>
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<tbody>
<tr>
<td>Plant biocides</td>
</tr>
<tr>
<td>Pyrrolizidine alkaloids</td>
</tr>
<tr>
<td>e.g., Crotalaria causing VOD</td>
</tr>
<tr>
<td>Cycasin and other β-glycosides</td>
</tr>
<tr>
<td>Cattle hepatotoxicity, Pacific region</td>
</tr>
<tr>
<td>Indospicine</td>
</tr>
<tr>
<td><em>Indigofera spicata</em> (“creeping indigo”), hepatotoxic to animals in Sri Lanka</td>
</tr>
<tr>
<td>Furanosesesquiterpenes</td>
</tr>
<tr>
<td>Myoporaceae, sheep, Australasia</td>
</tr>
<tr>
<td>Tetradymol</td>
</tr>
<tr>
<td><em>Tetradymia glabrata</em> (“horsebush”), sheep, western USA</td>
</tr>
<tr>
<td>Mycotoxins</td>
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<tr>
<td>Aflatoxin</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
</tr>
<tr>
<td>Lupinosis</td>
</tr>
<tr>
<td>Veterinary, lupinus contaminated with <em>phomopsis rossania</em></td>
</tr>
<tr>
<td>Environmental contaminants</td>
</tr>
<tr>
<td>Polycyclic aromatic hydrocarbons</td>
</tr>
<tr>
<td>Induction of cytochrome P-448: e.g., benzopyrene in charcoal-broiled meat, dioxin</td>
</tr>
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</table>

The hepatotoxicity of pyrrolizidine alkaloids is affected by nutritional status. Veno-occlusive disease (VOD) affected malnourished children, and, in the rat, a low-protein diet enhanced the acute hepatotoxicity of laisiocarpine. A high-casein diet increased survival in retrorsine-treated rats, but this longer survival permitted development of liver tumors.
ease" must include changes in liver function and morphology in malnutrition; altered hepatic handling of xenobiotics (Table 2); relationship between liver and other systems in malnutrition, particularly the gut and immune system; genetic variability in susceptibility to the effects of suboptimal nutrition (Table 3); and liver disease as a cause of malnutrition (Table 4).

This review attempts to evaluate recent data on some of these interactions. The temptation to draw a flow chart showing these interactions has been resisted. In interpreting recent literature, there are three reasons for caution.

1. Many data have been gained from the rat, which we know to be an imperfect model of the child. Examples of the way in which the rat has led us astray include the belief that choline was a lipotrope and that hepatocytes showed secretory-component-mediated uptake of IgA. Effects reported from animal studies are often short-term. Such experiments teach us about mechanisms but must not be incautiously extrapolated to chronic protein-energy malnutrition (PEM).

<table>
<thead>
<tr>
<th>TABLE 3. Genetic variability in susceptibility to liver damage</th>
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<tbody>
<tr>
<td>Susceptibility to fasting</td>
</tr>
<tr>
<td>Defects in gluconeogenesis</td>
</tr>
<tr>
<td>Defects in fatty acid oxidation</td>
</tr>
<tr>
<td>Pharmacogenetics</td>
</tr>
<tr>
<td>Acetylator status (isoniazid)</td>
</tr>
<tr>
<td>Immunogenetics</td>
</tr>
<tr>
<td>HLA B8 DW3 and chronic active hepatitis</td>
</tr>
<tr>
<td>Modulators of inflammation</td>
</tr>
<tr>
<td>α-1 antitrypsin phenotype</td>
</tr>
<tr>
<td>Heterozygosity</td>
</tr>
<tr>
<td>?Hemochromatosis</td>
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<table>
<thead>
<tr>
<th>TABLE 4. Liver disease as a cause of malnutrition*</th>
</tr>
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<tbody>
<tr>
<td>Parenchymal</td>
</tr>
<tr>
<td>Anorexia</td>
</tr>
<tr>
<td>Raised BMR, fever, hyperdynamic circulation</td>
</tr>
<tr>
<td>Dietary management of portasystemic encephalopathy (PSE)</td>
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<tr>
<td>Protein restriction</td>
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<tr>
<td>Branched-chain amino acids</td>
</tr>
<tr>
<td>Cholestasis</td>
</tr>
<tr>
<td>Fat malabsorption</td>
</tr>
<tr>
<td>Fat-soluble vitamins</td>
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</table>

*Liver disease also affects immune function, e.g., there is evidence that biliary IgA antibodies to enteric organisms are derived from plasma cells in the liver (9).
2. Single nutrient deficiencies or excesses are easy to contrive in the laboratory but rather rare in humans. Having said that, they are more likely to occur in situations of deprivation, where there is a single food source, than they are in the affluent, who eat food from a wide variety of sources.

3. Protein deficiency continues to be used as a model of PEM.

**FATTY LIVER IN KWASHIORKOR**

Possible mechanisms contributing to triglyceride accumulation within the hepatocyte are (a) increased delivery of fatty acids to the liver, (b) impaired beta oxidation, (c) impaired lipoprotein export, and (d) hepatocyte toxicity.

**Increased Delivery of Fatty Acids to the Liver**

Mobilization of free fatty acids (FFA) from peripheral tissues, by the glucose fatty acid cycle and by reduced serum insulin, is an appropriate response to starvation. Fatty acids are rapidly taken up from albumin by the liver [the "albumin receptor model" (10)]. Fatty acids taken up by the hepatocyte bind to a 12,000-dalton cytosolic protein [fatty acid binding protein (FABP)], which facilitates their diffusion through the cytosol to the membrane-associated enzymes of oxidation and esterification. Following esterification to fatty acyl CoA, there occurs an important branch point. One pathway leads to acylglycerides and thence to very low density lipoprotein (VLDL) formation, the first enzyme in the pathway being glycerophosphate acyltransferase (GPAT). The other leads via carnitine palmitoyltransferase (CPT I) on the outer mitochondrial membrane to β-oxidation. The relative proportions of fatty acids metabolized to acylglycerides or oxidized vary inversely in different physiologic states, although the total flux through these two pathways is determined only by the rate of delivery of fatty acids to the hepatocyte. The relative fluxes are determined largely by the activity of CPT I, which, like many regulatory enzymes, shows both rapid and slower changes. Rapid changes are achieved by changes in the concentrations of substrate or of competitive or allosteric inhibitors. Longer lasting effects are achieved by covalent modification of enzyme protein, change in organelle membrane fluidity, or change in concentration of enzyme protein. For CPT I, rapid increase in flux in starvation results from increased substrate (fatty acyl CoA) and reduced concentration of inhibitor (malonyl CoA). Starvation also introduces slower changes in the enzyme, an increase in its V\text{max}, and a reduction in its sensitivity to malonyl CoA inhibition. Grantham and Zammit (11) studied the recovery of CPT-I activity on refeeding rats. By 24 hr, the sensitivity to malonyl CoA inhibition was largely restored, but V\text{max} was not significantly changed. For this enzyme, there is some evidence that membrane fluidity may be the cause of the slower changes, since outer and inner mitochondrial membrane CPT activities correlate with each other and with β-oxidation rate, and inversely with mitochondrial membrane polarization (12).
Thus, in starvation, fatty acids will follow the route toward β-oxidation rather than that toward lipoprotein synthesis, so simply overloading the hepatocyte with fatty acids leads to ketogenesis and not fat accumulation, unless the maximal β-oxidation capacity is overwhelmed.

Carnitine deficiency is a theoretical cause of impaired mitochondrial fatty oxidation, but there is no evidence that diets lacking lysine, the precursor of carnitine, cause lowered plasma carnitine (13). In infancy, carnitine-free formulas lead to biochemical, though not clinical, deficiency. The need for carnitine in growing muscle may explain the difference between infants and adults. "Carnitine deficiency," as a cause of Reye's syndrome, probably represents one of a number of disorders of fatty acid oxidation. Mice treated with the carnitine analogues DL-aminocarnitine and acetyl-DL-aminocarnitine showed liver triglyceride accumulation only if starved (14).

Impaired β-Oxidation

Does the fatty liver of kwashiorkor result from impaired mitochondrial catabolism of fatty acids? A number of inherited metabolic abnormalities are associated with impaired β-oxidation, may present clinically with an illness like Reye's syndrome, and histologically show macro- and microvesicular fat in the liver cell (Table 5). Medium-chain acyl dehydrogenase (MCAD) deficiency appears to be the most common of these and the phenylpyruvic acid test is suggested as a screening procedure.

A similar pattern of micro- and macrosteatosis is seen in valproate toxicity, margosa oil poisoning, and Jamaican vomiting sickness. In these and in the inherited disorders, impaired β-oxidation is indicated by dicarboxylic aciduria.

<table>
<thead>
<tr>
<th>Reye's syndrome</th>
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<tbody>
<tr>
<td>Fatty acid oxidation defects</td>
</tr>
<tr>
<td>Medium-chain acyl dehydrogenase deficiency</td>
</tr>
<tr>
<td>Carnitine palmitoyl transferase deficiency</td>
</tr>
<tr>
<td>Multiple acyl dehydrogenase deficiencies</td>
</tr>
<tr>
<td>Severe: glutaric aciduria type II</td>
</tr>
<tr>
<td>Mild: ethylmalonic-adipic aciduria</td>
</tr>
<tr>
<td>Hydroxymethylglutaryl coenzyme A lyase deficiency</td>
</tr>
<tr>
<td>Urea cycle defects</td>
</tr>
<tr>
<td>Toxic</td>
</tr>
<tr>
<td>Jamaican vomiting sickness</td>
</tr>
<tr>
<td>Margosa oil intoxication</td>
</tr>
<tr>
<td>Sodium valproate</td>
</tr>
<tr>
<td>Salicylates</td>
</tr>
<tr>
<td>Multiple hornet stings</td>
</tr>
<tr>
<td>Alpers' disease (progressive neuronal degeneration of childhood with liver disease)</td>
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TABLE 5. Causes of microvesicular fatty change (15)
A much studied animal model is the weanling rat with riboflavin deficiency. Reduced activity of the three acyl-CoA flavoprotein dehydrogenases, short, medium, and long, causes fatty liver. Riboflavin deficiency is common in malnourished children. However, Ross and Hoppel (16) found that starvation partially reversed the reduction in mitochondrial fatty oxidation induced by riboflavin deficiency. This was associated with an increased activity of acyl-CoA dehydrogenase, suggested to be due to increased transport of the enzyme from cytosol to mitochondrion.

Defective β-oxidation seems unlikely to be a contributing factor to fatty liver in malnutrition because (a) the pattern of fat accumulation is macro- rather than microvesicular, and (b) dicarboxylic aciduria has not been noted.

Impaired Lipoprotein Export

Newly synthesized triglycerides and cholesterol are packaged with apoproteins B-100, C1, CII, CIII, and E to form VLDL for export from the hepatocyte. Does failure of apoprotein synthesis contribute to fatty liver?

Lowered plasma concentrations of albumin and other serum proteins are attributed to reduced hepatic synthesis. Protein depletion in the rat accordingly causes (a) reduced liver weight; (b) reduced hepatic DNA, RNA, protein, and free amino acids; (c) reduced RNA polymerase; (d) reduced polyribosome activity; (e) polyribosome disaggregation; and (e) reduced total mRNA. The availability of cDNA probes for individual rat proteins now enables specific mRNA concentrations, and hence rates of synthesis, to be measured for individual proteins. Thus, after 48 hr of fasting, mRNA levels of apolipoprotein E, apolipoprotein A-IV, albumin, transferrin, and transthyretin (pre-albumin) decreased by 15 to 30%, while apolipoprotein A-II decreased by 78% (18,19). These experiments have shown that the concentrations of mRNA are individually controlled. The mechanism of reduction of mRNA remains to be elicited. Is it reduced transcription, reduced rate of delivery of newly synthesized mRNA to the cytosol, reduced rate of mRNA degradation, or increased translational efficiency? Early work suggested that amino acids, particularly tryptophan, caused a rapid return of mRNA levels and protein synthesis to normal after fasting, suggesting that tryptophan facilitated mRNA export from nucleus to cytosol. This is not confirmed in more recent work, in which mRNA values were unchanged after 4 hr of refeeding with casein supplemented with tryptophan and arginine but returned to normal after 3 days of a 30% protein diet. Sakuma et al. (20) confirmed that a low-protein diet indeed reduced albumin mRNA but found that a low-protein, low-energy diet failed to do so.

The time is now ripe for a study of mRNA synthesis in clinical malnutrition using the techniques of in situ hybridization and available cDNA probes. Rat models of failure of VLDL export include orotate administration, which causes an inability to glycosylate apoproteins, and deficiency of the "lipotrophic agents"—choline, methionine, and inositol. These models are not clinically relevant (1).
Toxic Damage to the Cell

Fatty change is a feature of toxic liver damage caused by carbon tetrachloride, alcohol, and drugs such as methotrexate, perhexaline maleate, amiodarone, and chloroquine (21). Carbon tetrachloride hepatotoxicity is mediated by the formation of reactive free radicals, which react with macromolecules, in particular unsaturated fatty acids in organelle membranes. The toxic free radical theory for the etiology of kwashiorkor (4) postulates that various noxious stimuli lead to free radical production, which, in the presence of inadequacy of protective mechanisms and increased hepatic iron, produces lipid peroxidation.

In the mitochondrial reduction of oxygen to water, the cytochrome oxidase complex achieves the addition of four electrons to oxygen without the formation of intermediate toxic pre-radicals. A small fraction of oxygen, however, escapes this control pathway and is reduced by single electron addition, as, for example, by cytochrome P-450-dependent mixed function oxidases. The first stage in this is generation of the superoxide free radical:

\[ \text{O}_2^- + e^- \rightarrow \text{O}_2^{2-} \]

Superoxide yields the highly reactive hydroxyl radical in the Haber-Weiss reaction:

\[ \text{O}_2^{2-} + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \text{OH}^- + \text{OH}^- \]

The rate constant for this reaction in aqueous solution is very slow, however, unless it is catalyzed by a transitional metal as follows (the Fenton reaction):

\[ \text{Cu}^{2+} + \text{O}_2^{2-} \rightarrow \text{Cu}^+ + \text{O}_2 \]
\[ \text{Cu}^+ + \text{H}_2\text{O}_2 \rightarrow \text{Cu}^{2+} + \text{OH}^- + \text{OH}^- \]

The hydroxyl radical has a very short half-life, measured in milliseconds, and reacts with other molecules in its immediate environment. Other secondarily generated free radicals may be both less toxic and more mobile. Damage induced by hydroxyl ion generation may occur both to macromolecules such as DNA, protein, and nucleotides, and also to membranes. The best studied chain free radical reaction is lipid peroxidation, in which unsaturated fatty acids within membranes are oxidized, leading to such membrane alterations as change in fluidity, change in receptor activity, and change in enzyme activity. Protective enzymatic mechanisms remove superoxide (superoxide dismutase), hydrogen peroxide (catalase), and other secondarily oxidized species (glutathione peroxidase).

A diet deficient in vitamin E and selenium produces fatal hepatic necrosis in the rat. Fraga et al. (22) recently evaluated the role of vitamin E and selenium as protective agents against oxidative stress by measuring liver chemiluminescence in situ. Weanling rats fed a diet deficient in vitamin E and selenium showed liver chemiluminescence that was increased 60 and 100% over control values. The double deficiency led to hepatic necrosis, though single deficiencies in either vitamin E or
selenium did not produce liver necrosis but increased liver chemiluminescence. The activity of liver selenium-glutathione peroxidase diminished to 13% of control value in rats fed selenium-deficient diets.

It is, however, unlikely that dietary deficiencies of tocopherol and/or selenium alone will cause liver necrosis in humans. The only recognized clinical association with selenium deficiency is Keshan disease, a cardiomyopathy of children and young women in China (23). Evidence is accumulating, however, that hepatic Cu-Zn and Mn-superoxide dismutase, glutathione peroxidase, and glutathione are reduced in kwashiorkor, leaving the hepatocyte vulnerable to oxidative stress.

Noxious Stimuli Causing Oxidative Stress

Aflatoxin

Aflatoxins (AFB1) are metabolites of Aspergillus flavus and closely related fungi and are frequent contaminants of many foods in situations where handling and storage facilities are inadequate. High levels are found in the liver of children with kwashiorkor in Sudan, South Africa, Nigeria, and Ghana (4,24). AFB1 is metabolized by the mixed function oxidase system to a number of metabolites including AFB1-2,3, epoxide, a highly reactive molecule.

Aflatoxin may be the prototype of many mycotoxins capable of producing oxidative damage to the nutritionally vulnerable hepatocyte.

Gut Derived Toxins

1. Infection is a common precipitant of kwashiorkor, in particular chronic diarrhea responsive to metronidazole and associated with small bowel bacterial contamination. Intracellular killing of bacteria by neutrophils employs a respiratory burst, which generates free radicals.
2. Small bowel contamination causes bacterial deconjugation of bile acids, yielding the toxic monohydroxy lithocholic acid. Its toxicity is attested to in the experimental animal and in studies of total parenteral nutrition (TPN) in adults.
3. Bacterial sepsis is associated with cholestasis, particularly in the newborn but also in older patients (25).
4. Endotoxin has an important role in a number of situations of liver damage. Endotoxin plays an important role in experimental liver injury produced by choline deficiency, carbon tetrachloride, and galactosamine (1).

Bowel Wall Permeability

Udall et al. (26) suggested that permeability of the neonatal gut to proteases, both bacterial and pancreatic, may be important in the liver disease of α-1 antitrypsin de-
ficiency. If malnutrition increases mucosal permeability, increased portal vein endotoxin, secondary bile acids, and proteases may be hepatotoxic.

**LIVER DAMAGE FROM EXCESS ENERGY INTAKE**

In a symposium on malnutrition, it is appropriate briefly to mention evidence that energy excess is also hepatotoxic.

**Steato-Hepatitis in Obesity**

Grossly obese adults may have abnormal liver function tests and biopsies showing fatty change, hepatitis, fibrosis, and occasionally cirrhosis. Although this is poorly documented in childhood, Moran et al. (27) describe three fat adolescents in whom abnormal liver function tests were accompanied by fatty change and mild portal tract changes. Animal models of this may be the following: (a) *the 'fat cow syndrome'* (the parturition, or fatty liver syndrome, of dairy cows). In the early weeks of lactation, approximately one-third of high-yielding dairy cows may develop subclinical fatty liver. In the immediate postpartum period, cows who had, until parturition, consumed a high-energy diet, enter a phase of negative energy balance of the order of 30 MJ/day (28). Serum concentrations of free fatty acids and β-hydroxybutyrate indicate fatty acid mobilization, and massive fat deposition occurs in liver, kidney, muscle, and adrenal cortex. However, there is no evidence that this severe example of fatty liver ever proceeds to chronic liver disease. (b) *Fatal fatty liver-kidney syndrome in obese monkeys.* Laber-Laird et al. (29) describe a syndrome affecting adult female obese cynomolgus monkeys in which sudden death follows a brief period of anorexia. A diffuse fatty infiltration of liver and kidneys is found. Similar syndromes are described in guinea pigs and cats and, like fatty liver of pregnancy in women, are of unknown etiology.

**Liver Damage Following Intestinal Bypass**

Following jejunal ileal bypass for morbid obesity, liver failure occurs in approximately 5% of patients and liver dysfunction in 45% (30). A number of animal experiments incriminate the excluded limb of bowel. For example, Hollenbeck et al. (31) produced a jejunoileal bypass in dogs. In animals that did not receive antibiotics, anaerobic culture from the defunctionalized limb grew *Bacterioides* species: all animals died from liver failure within 122 days of the procedure. Treatment with doxycycline hyclate prevented bacterial overgrowth and allowed survival to the end of the study. Resection, rather than bypass, of 80% of the small bowel, also in the dog, achieved weight loss without hepatic injury. Similar studies in the rat demonstrated...
that antibiotics significantly reduced the fatty deposition within the liver after jejunooileal bypass.

**Energy Restriction and Longevity**

Energy restriction in rats promotes a longer life span and delays the onset of malignancies and other diseases of later life (32). Among possible explanations are reduced production of reactive free radicals and increased catalase activity. Contrary to some earlier work, Frederiks et al. (33) found that 24-hr fasting reduced the hepatic damage of liver produced by 60 min of ischemia.

**Xenobiotics**

Rats rendered obese by overfeeding and given 710 mg/kg paracetamol intraperitoneally showed more liver damage than controls, as judged biochemically or histologically. This difference persisted when peak plasma levels were matched (34).

**ONCOGENESIS**

The evidence linking primary hepatocellular carcinoma (HCC) and hepatitis B virus (HBV) is overwhelming. HBV-related HCC is associated with the chronic carrier state and in particular with the incorporation of HBV DNA into the host genome. The risk of acquiring hepatitis B surface antigen (HBsAg) chronic carriage following adult infection is of the order of 5%, but greater than 90% in babies born to hepatitis Be antigen (HBeAg)-positive carrier mothers. We may speculate that malnutrition, by impairing the cellular immune response, might increase the risk of chronic carriage and hence HCC. There is no evidence that this is actually so, and indeed, it seems likely that the high prevalence of carrier babies results from blocking of T-cell attack on infected hepatocytes by passively transferred maternal IgG anti-hepatitis B core (HBc) (35,36).

Aflatoxin may act synergistically with HBV or alone to produce HCC. Experimentally, however, a higher protein intake enhances the development of AFB-related HCC. A protein intake in excess of that required to sustain maximum growth rate in rats favors the development of gamma-glutamyl transpeptidase-positive foci, believed to be preneoplastic lesions (37). On the other hand, an acute fast appears to enhance the growth of tumors in rats, with a FFA and/or ketone body availability being suggested explanations (38). Chemical carcinogenesis is promoted by choline and methionine deficiency in rats, though apparently not in woodchucks (or humans) (39).

Oncogene expression and malnutrition have yet to be evaluated. Oncogenes control cell proliferation, differentiation, and tumorigenesis, encoding proteins that in-
clude growth factors, protein kinases, and nuclear proteins, which may regulate
gene expression (40). Horikawa et al. (44) studied rats in whom protein deprivation
for several days was followed by a protein meal to induce DNA synthesis. They
found that mRNA for c-myc oncogene gradually rose four- to fivefold during protein
depprivation, and fell rapidly when protein was given. It is likely that oncogenesis re-
quires activation of more than one cellular oncogene and loss of other suppressor
genes.

SPECIFIC DIETARY EXCESS

Vitamin A

Hypervitaminosis A causes perisinusoidal fibrosis, terminal hepatic vein sclero-
sis, and focal congestion. Vitamin A is stored in Ito cells or lipocytes, which on vi-
tamin A overdosage, are filled with fat and show fluorescence. Morphologically,
they are seen to be associated with new collagen synthesis and, indeed, to change
into fibroblasts (42).

In the presence of malnutrition and lowered serum retinol binding protein, serum
vitamin A levels may be a poor guide to hepatic vitamin A status, and the relative
dose response may be a more effective way of judging vitamin A deficiency (43).
The occurrence of severe hypervitaminosis A in two siblings (associated with
chicken liver spread) suggests a familial sensitivity to vitamin A (44).

Vitamin E

An intravenous vitamin E preparation (E-Ferol) in premature infants produced an
unusual symptom complex comprising pulmonary deterioration, thrombocytopenia,
liver failure, ascites, and renal failure.

Copper

Unlike kwashiorkor, Indian childhood cirrhosis is associated with very high he-
patic concentrations of copper, which derive from untinned brass utensils used for
early infant feeding. That this copper is hepatotoxic is suggested by (a) the preven-
tion of Indian childhood cirrhosis (ICC) by preventing this infant feeding practice,
and (b) successful treatment of ICC with penicillamine (45-48). In the rat, how-
ever, excess dietary copper fails to produce cirrhosis, and indeed ameliorates liver
damage induced by galactosamine or carbon tetrachloride (49). This leads to the
suggestion that ICC may result from synergistic toxicity between copper and a plant
or biocide (8). This would be a situation very similar to siderosis, in which alcohol
is synergistic with iron in producing cirrhosis.
ACKNOWLEDGMENT

Financial support from the Wellcome Trust and the Michael McGough Foundation for original work referred to in the text is acknowledged.

REFERENCES


**DISCUSSION**

*Dr. Suskind:* Could you explain the way in which aflatoxin may be a factor in the development of protein-energy malnutrition (PEM)?

*Dr. Tanner:* Although aflatoxin has been found in the liver of children with kwashiorkor, care is needed in attributing a causal role to it. First, urinary aflatoxin metabolite concentrations appear equally high in kwashiorkor and marasmus. Second, methodologic difficulties in measuring aflatoxin have impaired epidemiologic studies of aflatoxin exposure. It is hoped that the newer *Enzyme-Linked ImmunoSorbent Assay* (ELISA) techniques will make it pos-
sible to determine how widespread exposure to aflatoxin is (1). Third, aflatoxin may be just one of a number of mycotoxins and other toxins contributing to liver injury. Fourth, the child with kwashiorkor may be less able to metabolize the toxic epoxy derivatives of aflatoxin and, thus, be more susceptible to the harmful effects of an almost universal exposure.

**Dr. Jackson:** Can you explain why there was a response to 30% protein, but no response to amino acids, in the study using cDNA probes? Also, could you expand on the suggestion that there is a male predisposition for both cirrhosis and Indian childhood cirrhosis (ICC)? Finally, in the production of fatty liver, there may be different distributions of fat. Does the pattern of distribution offer any guidance to possible differences in etiology?

**Dr. Tanner:** The response to amino acids was studied for 30 min to 4 hr by de Jong and Schreiber (2). Earlier work by others had shown that a single tube feeding of tryptophan, or a complete amino acid mixture, increased protein synthesis in the fasted rat by increasing the translocation of poly(A)-mRNA from the nucleus to the cytoplasm (3). de Jong and Schreiber attribute the differences in their results to the fact that, in the earlier studies, rats were acutely fasted rather than protein-depleted for 3 days. The response to protein refeeding may be transcriptional and/or translational.

Second, the male disposition for ICC may relate to exposure to copper-contaminated animal milk but does, at least partially, result from a genuine sex difference in susceptibility. Older siblings of cases of ICC who escaped the disease are more likely to be female than male, and those girls who do get the disease seem to have had a higher copper intake than the boys (4). Certainly, a difference in susceptibility to xenobiotic toxicity may be relevant, as suggested by sex differences in susceptibility to pyrrolizidine alkaloid toxicity, both in the experimental rat and clinically in veno-occlusive disease.

The question of distribution of fat is interesting. It is important, of course, to distinguish between microvesicular and macrovesicular fatty deposition. We are now referring only to the latter. The largely centrilobular distribution of fat in carbon tetrachloride hepatotoxicity may represent zonation associated with reducing oxygen tension along the sinusoid. One might, conversely, expect more periportal fatty change in response to toxic substances reaching the liver via the portal vein.

**Dr. Jackson:** You supported the theory that fatty liver in malnutrition might be caused by free radical toxicity by drawing a parallel with carbon tetrachloride. Since, in malnutrition, you start off with periportal deposition, I am not sure how you came to that conclusion.

**Dr. Tanner:** The centrilobular fatty change of carbon tetrachloride toxicity is associated with production of the relevant free radical by cytochrome P450-related activity in those centrilobular hepatocytes. Other toxins that might act via free-radical mechanisms may be activated in the periportal zones, but I agree that an accurate analogy will depend on further understanding of the zonal distribution of pathology.

**Dr. Warrier:** I question the explanation of copper pots causing ICC. I believe most people use aluminum, not brass, and do not feed males preferentially over females. In addition, I suspect that aflatoxin is just one piece of the multifactorial attack on the liver.

**Dr. Tanner:** The only rigorous piece of work in ICC is a study implicating the preparation of infant milk feed in brass, not copper, utensils. Concerning male predominance, I believe there is a genuine male susceptibility in addition to a possible difference in feeding practices.

Regarding aflatoxin, a recent paper speculates on the role of plant biocides and mycotoxins in ICC (5); the fact remains that if copper ingestion is prevented, ICC is prevented. What we must continue to deal with are all the other cirrhoses of Indian childhood with which ICC has, unfortunately, been confused.

I should like to mention, also, the work of Dr. Parekh and Dr. B.D. Patel on alkaline phos-
phatase isoenzymes, although I am not sure whether the presence of the Regan isoenzyme points to primary genetic susceptibility or is a marker of toxic hepatocyte damage.

With regard to reports of copper toxicosis in other parts of the world, a number of isolated case reports describe an entity that is probably familial, may have nothing to do with copper ingestion, and affects children slightly older than the typical case of ICC, but is associated with high-level copper and a histology very similar to ICC (6-9).

**Dr. M. Mehta:** We should note that ICC has also been found where brass utensils have not been used and where there was no indication of sex preference.

**Dr. Guesry:** Sixty years ago, all milk was processed in brass containers. Do you know the incidence, at that time, of liver cirrhosis in infants fed with dry milk?

**Dr. Tanner:** I should suspect that those brass containers were, in fact, tinned. Tinning of brass utensils, still done in rural India, is an effective way of preventing copper uptake from casein chelation.

**Dr. Durie:** In any form of cholestasis there is a high liver content of copper. Would you please comment on that in relation to ICC?

Dr. Sokol (10) in Denver has shown that vitamin deficient rats, if given an excess of dietary copper, develop severe liver disease. Would you comment on the concept of free radicals in relation to copper and vitamin E in liver disease?

**Dr. Tanner:** The best control group for ICC with regard to cholestasis is, of course, extrahepatic biliary atresia. Infants with biliary atresia in India have profound cholestasis from an early age and live in the same environment as cases of ICC. Children with biliary atresia seen in Pune do, indeed, show the expected copper accumulation, but not to the extent seen in ICC. Furthermore, in ICC, cholestasis is a very late feature, both clinically and histologically.

I am very interested in the study showing that vitamin E deficient rats develop severe liver disease if given copper. This supports the concept that impairment of protective mechanisms may allow heavy metal–induced free radical toxicity to become manifest and is in accordance with the studies suggesting that vitamin E or selenium deficiency alone produces little liver damage but together may produce acute hepatic necrosis.

**Dr. Truswell:** In addition to considering the effect of malnutrition on drug metabolism, we should also be investigating what drugs can do to the state of nutrition (11,12). We know, for example, that there are drugs such as tetracyclines that interfere with protein synthesis.

A second question concerns drug binding. I guess that if a child is malnourished with marasmus, as long as doses are based on body weight rather than age, the doses of the drugs might not be too far off. But if the child has a relatively preserved body weight but a very low plasma albumin, of about 1 g/ml, then there might be trouble with drugs such as barbiturates, dioxins, and salicylates.

Buchanan (13) did a number of studies on in vitro drug binding in PEM that should be mentioned. There is nothing worse than giving thiopentone anesthesia, which is normally bound to plasma albumin, to a child with very low plasma albumin.

**Dr. Tanner:** It is important, in computing doses for marasmic children, to remember differences in body water distribution and the lack of fat. With regard to endotoxemia, I suspect that endotoxin is one part of a multifactorial attack on the liver.

**Dr. Ballabriga:** In relation to the etiology of the fatty liver infiltration, the fatty acid composition will change according to the composition of the intake. If intake contained high quantities of vegetable oil rich in polyunsaturated fatty acids, there may be a lack of vitamin E, and lipid peroxidation of these fatty acids may increase.

We studied fatty acid composition of the main phosphoglycerides in the human liver in
newborns. The groups were made up of infants who had died during the first day of life before receiving any food and infants who had died during the first month of life after receiving parenteral nutrition with 2 g of Intralipid/kilo per day for about 3 weeks. Fat infusion had a high content of linoleic acid.

During normal maturation, liver and brain fatty acid composition show a progressive increase of the fatty acids of the family n3 of the main phosphoglycerides. If, during parenteral nutrition, large quantities of linoleate are given, the natural preference of the 6-desaturase system by the n3 family will be overcome and displaced toward the predominant n6 fatty acids (14).

Dr. Jackson: Most solutions for TPN contain little or no cysteine, and the methionine they contain is not converted to cysteine very effectively. One important difference between the fatty liver produced by limited availability of methyl groups, including choline deficiency, and that produced by a low-protein diet is that, in the latter, there is a change in the rate of clearance of xenobiotics (15). This is presumed to relate to a change in the activity of the glutathione-S transferase series of enzymes and possibly also the availability of glutathione (16). This may be one important variable that provides a link between the development of fatty liver due to a nontoxic dietary manipulation and the decreased resistance to free radical-induced damage. This would provide increased susceptibility to any additional toxic component that would promote the development of fatty liver, a final common pathway for a range of insults.

Dr. Tanner: Clearly, we need to study fatty acids in membrane lipids as well as in the globular fat in the cell.

In addition, the study of lipotropes is becoming fashionable in relation to oncogenesis. A diet low in methionine or choline does promote the formation of tumors caused by other carcinogens, and prolonged administration of a deficient diet causes the development of hyperplastic nodules.

REFERENCES


