In the 1960s, my lab was interested in understanding how bilirubin and other organic anions are transferred from the plasma through the liver cell and into the bile. We performed gel filtration of liver supernatants and identified two protein fractions, designated Y and Z, which bound organic anions including bilirubin, and thus we proposed that they were involved in hepatic uptake of organic anions from plasma. Subsequently, the Y and Z fractions responsible for this binding activity were purified, cloned, and sequenced. Y was identified as a member of the glutathione S-transferase (GST) protein family and Z found to be a member of the fatty acid–binding protein (FABP) family. These proteins have since been shown to have additional surprising roles, but understanding of their full role in physiology and disease has not yet been achieved.

In the 1960s, bilirubin metabolism was a “hot” topic. Along with other groups, my lab was studying various forms of inheritable jaundice in an effort to dissect the mechanism of bilirubin's transfer from plasma into the hepatocyte and its role in intracellular metabolism and biliary secretion. These processes were eventually identified and found to be related to the basic mechanisms whereby the liver handles many anionic drugs, metabolites, and hormones. Because the mechanism of hepatic uptake of bilirubin was unknown, A.J. Levi, Z. Garma, and I took advantage of advances in gel permeation chromatography to study this process. In 1969, we described two hepatic cytoplasmic protein fractions, designated Y and Z, that bound bilirubin and various dyes in vivo and in vitro and, based on tissue distribution, abundance, and effects of genetic and pharmacologic models, were proposed to participate in organic anion uptake (1).

In the decades since then, the Y and Z proteins have been identified as members of large protein families that were cloned and sequenced. Several surprising functions emerged, whereas others are proposed based on binding properties. Many challenges remain in understanding the full role of these proteins in physiology and disease.

**Liver function from Y to Z**

Irwin M. Arias

NIH, Bethesda, Maryland, USA.

In the 1960s, my lab was interested in understanding how bilirubin and other organic anions are transferred from the plasma through the liver cell and into the bile. We performed gel filtration of liver supernatants and identified two protein fractions, designated Y and Z, which bound organic anions including bilirubin, and thus we proposed that they were involved in hepatic uptake of organic anions from plasma. Subsequently, the Y and Z proteins responsible for this binding activity were purified, cloned, and sequenced. Y was identified as a member of the glutathione S-transferase (GST) protein family and Z found to be a member of the fatty acid–binding protein (FABP) family. These proteins have since been shown to have additional surprising roles, but understanding of their full role in physiology and disease has not yet been achieved.
Acids was demonstrated resulting in a name change to fat acid–binding protein (FABP) (10, 11). Because FABP did not participate in fatty acid uptake, a role in intracellular fatty acid utilization was proposed (10, 11).

The FABPs are an evolutionarily conserved family of nine approximately 14-kDa binding proteins for fatty acids and other lipophilic substances, such as eicosanoids and retinoids. The family contains liver, intestinal, heart, adipocyte, epidermal, ileal, brain, myelin, and testes FABPs; however, no FABP is completely tissue specific, and most tissues express several isoforms. In hepatocytes, adipocytes, and cardiac myocytes, where fatty acids are prominent species, FABPs account for 1%–5% of all cytoplasmic proteins, and their expression is further induced after mass influx of lipid (10, 11). Liver-FABP, but not ligandin, is induced by peroxisome proliferators that regulate liver-FABP through PPARs. The crystal structure of synthetic adipose-FABP revealed features of lipid-protein interactions that differ from those present in apolipoproteins and albumin (12). No enzymatic activity has been associated with any FABP.

Despite much research, little is known about the precise biological functions and mechanisms of action of FABPs. Their distribution and regulation suggest possible roles in lipid metabolism. FABPs are proposed to shuttle fatty acids to specific enzymes and cellular compartments, modulate intracellular lipid metabolism, regulate gene expression, and, through their binding of eicosanoids and retinoids, to be linked to metabolic and inflammatory pathways (13). However, most proposals are based on vitro studies. In vivo demonstration of the proposed functions is still lacking. Molecular knockout experiments in animals and cells reveal an altered phenotype only when lipid influx is greatly increased (13). A direct link between in vivo data gained from genetic knockout models of FABPs or PPARs remains inconclusive, possibly due to protein redundancy and compensatory mechanisms.

From humble beginnings
Thus, a relatively uncomplicated 1969 gel filtration study of rodent 100,000 g liver supernate fraction (Figure 1) prompted an unexpectedly wide range of discoveries relating to net hepatic organic anion uptake, glutathione metabolism and detoxification, multifunctional proteins, lipid metabolism, inflammatory pathways, and possibly azo dye carcinogenesis. Current research worldwide focuses on the role of GSTs in protein–protein interaction and signaling, oxidative stress, gene expression and regulation, and renewed interest in net hepatic uptake in physiology and disease. Studies of FABPs continue, particularly regarding their function and transcriptional and epigenetic regulation in metabolic stress, obesity, and other pathologic states. Determining the function of cytoplasmic binding proteins, such as Y and Z, particularly when there are many isoforms and gene products within a single cell, has proven to be a difficult challenge that we hope can be met using high-resolution live-cell imaging of fluorescently labeled ligands and candidate acceptor molecules.

Acknowledgments
The studies reported by Jonathan Levi in 1969 were continued by a large number of fellows and colleagues in our laboratory at the Albert Einstein College of Medicine and worldwide. After a distinguished academic career in London, Jonathan Levi died in 1998. This Hindsight article is dedicated to the memory of his scholarship and friendship.

Address correspondence to: Irwin Arias, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Section on Intracellular Protein Trafficking (SIPT), 18T Center Dr., Room 101, MSC 5430, Bethesda, Maryland 20892-5430, USA. Phone: 301.402.8394; Fax: 301.402.0078; E-mail: ariasl@mail.nih.gov.