LIVER DISEASES IN INFANCY AND CHILDHOOD

edited by

SAMUEL R. BERENBERG, M.D.

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1976
MARTINUS NIJHOFF MEDICAL DIVISION - THE HAGUE

ISBN 90.247.1921.6

Printed in the Netherlands.

PREFACE

For almost four decades the Josiah Macy, Jr. Foundation has been convening conferences relating to medicine, medical education, and health care in their broadest contexts. During the 1940s and 1950s the conferences focused on biomedical research, which was at that time in its golden age in the United States. As medical care and medical education ascended in importance, since the mid-1960s the conferences have been largely concentrated on topics in these fields.

The Macy Foundation also fosters international conferences, and a major effort in recent years has been a rewarding collaboration with France's most distinguished medical statesman, Professor Robert Debré, and the International Children's Centre in Paris, which he founded and directs. Nineteen seventy-five was an especially busy year for this Franco-American alliance: in April there was a seminar on 'The Family Doctor: France and the United States'; and, in June, a conference on 'Diseases of the Liver in Infancy and Childhood'. Earlier Franco-American conferences have been on 'Brain Development' (1972) and on 'Puberty' (1974).

As with the others the participants in this conference on Liver Disease in Infancy and Childhood were drawn from the United States and Europe. We were especially pleased to have as three of the participants men who hold sabbatical awards as Macy Foundation Faculty Scholars, Ivan Diamond, M.D., Ph.D., Thomas Starzl, M.D., Ph.D. and M. Michael Thaler, M.D.

Our continuing pleasure is the rewarding liaison with Professor Robert Debré who has made so many enduring contributions to medicine and health in France and elsewhere in the world.

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ABBREVIATIONS

ALG	=	antilymphocytic globulin
ALS	=	antilymphocytic serum
α-1 AT	=	α-1 antitrypsin
ATP	=	adenosine triphosphate
Brb	=	bilirubin
Brb - Alb	=	bilirubin - albumin
BSA	=	bovine serum albumin
BSP	=	sulfobromophthalein
CD	=	circular dichroism
CMV	=	cytomegalic virus
CNS	=	central nervous system
CoA	=	coenzyme A
CPS	=	carbamyl phosphate synthetase
DBSP	=	dibromsulfophthalein
DDT	=	1,1,1 Trichloro-2,2-bis (P-chlorophenyl ethane)
DNA	=	deoxyribonucleic acid
ER	=	endoplasmic reticulum
FSH	=	follicle stimulating hormone
GI	=	gastrointestinal
GSH transferase	=	glutathione transferase
H and E	=	hemotoxylin and eosin (stain)
HBABA	=	parahydroxybenzeneazobenzoic acid
HBcAb	=	hepatitis B core antibody
HbcAg	=	hepatitis core antigen
HBSAB	=	hepatitis B surface antibody
HBsAg	=	hepatitis B surface antigen
HBV	=	hepatitis B virus
HPC	=	hepatoporto/cholecystostomy

HPE	=	hepatoporto/enterostomy
HPS	=	hepatoporto/stomy
HSA	=	human serum albumin
ICG	=	indocyanine green
Ig	=	immune globulin
IgG	=	immunoglobulin class G
I.P.	=	intraperitoneal
K1, K2	=	first order rate constants for influx and efflux
L.D.	=	lethal dose
MHV	=	mouse hepatitis virus
NRS	=	normal rabbit serum
O.T.	=	orthoptic transplant
OTC	=	ornithine transcarbamylase
PAPS	=	3′ phosphoadenosine 5′ phosphosulfate
PAS	=	periodic acid Schiff (reaction)
QAE-A	=	quarternary aminoethyl cellulose ion exchange resin
RNA	=	ribonucleic acid
RSA	=	rat serum albumin
S	=	storage capacity
S.D.	=	standard deviation
SDS	=	sodium dodecyl sulfate
S.E.M.	=	standard error of the mean
SER	=	smooth endoplasmic reticulum
SGOT	=	serum glutamic oxaloacetic transaminase
SGPT	=	serum glutamic pyruvic transaminase
TCDD	=	tetrachlorodibenzodioxan
Tm	=	transport maximum
UDPG	=	uridine diphosphate glucose
UDPGA	=	uridine diphosphate glucuronic acid
UDPGT	=	uridine diphosphate glucuronyl transferase

HOMMAGE AU PROFESSEUR MARCEL LELONG
(1892–1973)

De 1928, où il était jeune chef de clinique de Lereboullet, jusqu'à 1963, où il quittait l'hôpital, Marcel Lelong a animé le vieil établissement parisien des 'Enfants Trouvés', devenu sous son impulsion le grand centre pédiatrique moderne qu'est encore l'hôpital Saint-Vincent-de-Paul. Pendant cette longue carrière vécue tout entière dans le même lieu, Marcel Lelong est devenu l'un des grands maîtres de la pédiatrie française, à la tête de la Clinique de Pédiatrie et de Puériculture, qui fut créée pour lui, au lendemain de la deuxième guerre mondiale.

Pendant les dix dernières années de sa vie hospitalière, il a su grouper autour de lui tout le prestige technique et humain que lui avait légué son maître Robert Debré dont il avait été le premier élève et dont il sut maintenir l'Ecole au niveau d'une remarquable activité dans le domaine de la connaissance, des soins et de la prévention des maladies de l'enfant. Ce que lui doit la médecine prénatale et périnatale de notre pays peut être mieux mesuré aujourd'hui où la néonatologie est enfin un secteur autonome de la pédiatrie.

La création de l'Ecole de Puériculture lui a permis d'implanter, en France et en Europe, les structures et les techniques qui ont longtemps servi de centre pilote dans ce domaine.

Son action médicale et sociale en faveur des enfants abandonnés lui a permis d'inspirer, à l'administration de l'Assistance Publique puis à l'Aide Sociale à l'Enfance, les solutions les meilleures pour le développement physique, mental et moral de ces enfants. Un grand nombre d'affections de l'enfance lui sont redevables de progrès dûs à la fois à la clinique et à la biologie car il savait associer ses observations raffinées faites au lit du malade et les investigations du laboratoire. Il apporta des connaissances nouvelles dans le domaine des maladies virales et bactériennes, parasitaires, des erreurs innées du métabolisme, des malformations congénitales, des troubles de la croissance et du développement, grâce à l'exercice d'une médecine traditionnelle excellente et aux recherches les plus récemment entreprises.

Novateur, animateur, grand chef d'Ecole, pédiatre universellement renommé, savant et homme de coeur, sa mémoire mérite d'être honorée et son nom donné par la Josiah Macy Jr. Foundation à ce séminaire voué à la pathologie hépato-biliaire de l'enfant.

CONTENTS

EXTRACELLULAR AND INTRACELLULAR TRANSPORT OF BILIRUBIN

IRWIN M. ARIAS, M.D.

Bilirubin is an orange organic anion of molecular weight 584 which is exclusively derived from the catabolism of heme protein. It has limited aqueous solubility at physiologic pH and when found in tissues is largely, if not exclusively, bound to protein or dissolved in lipid. At least 85% of bilirubin formed in man is derived from the systematic degradation of mature circulating erythrocytes as a function of their age. Conversion of heme to bilirubin in the reticuloendothelial cells is quantitative and apparently limited by activity of the microsomal heme oxygenase system. Bilirubin is also derived from erythropoietic sources and degradation of heme proteins, particularly liver heme proteins such as cytochrome P-450 because of its abundance and relatively short half-life. Whether bilirubin derived from these various sources enters the same body pools and participates in similar transport mechanisms in the liver is unexplored.

Bilirubin does neither good nor harm with exception of the risk of kernicterus in neonates and rare situations of lifelong profound unconjugated hyperbilirubinemia in adolescents. In pediatrics, the major emphasis has been on understanding the metabolism and regulation of this potential brain toxin; however, one should recognize that such studies may be more important for the information they yield regarding the transport of other organic anions of greater physiologic importance than bilirubin... such as various drugs and metabolites. Many of the transport processes utilized by bilirubin in its transfer from plasma into the bile are shared by other substrates which often alter hyperbilirubinemia in various ways.

The underlying basis of jaundice in neonates is the 'physiologic' hyperbilirubinemia seen in virtually all newborn infants. During the past 20 years, studies of this entity have had as their premise that understanding of the rate-limiting step resulting in 'physiologic' jaundice will permit proper therapeutic approaches to reduce unconjugated bilirubin when it accumulates in excess in the plasma and tissues of neonates and increases the risk of kernicterus (1). Of necessity much of this work has involved laboratory animals

which introduces interpretative difficulties when such results are applied to man. The numerous studies of neonatal jaundice have not identified the rate-limiting step and have only emphasized our limited knowledge of the complexity of the liver in the neonate. Numerous qualitative observations have been made but to date there is no quantitative assessment of these parameters in man.

Theoretically, 'physiologic' jaundice can result from one or more of the mechanisms: 1. increased bile pigment production from hemolysis or sources other than mature, circulating erythrocytes; 2. impaired transfer of bilirubin from plasma to the site of glucuronide formation; 3. impaired formation of bilirubin glucuronide due to deficient or inhibited UDPGA formation or UDPGT activity; and 4. increased intestinal absorption of bilirubin after biliary excretion and hydrolysis of bilirubin glucuronide.

At present, the pathophysiology of 'physiologic' jaundice is considered to involve transient developmental deficiency of UDPGT. Increased production or intestinal reabsorption of bilirubin may be associated phenomena but are not capable of producing jaundice alone. The following summarizes evidence in support of this hypothesis (1):

1. In several animals, including primates, UDPGA formation and UDPGT activity are virtually absent in fetal liver and attain adult levels between the second and tenth day of life.

2. UDPGT is associated with the microsomal fraction of liver homogenates and electron microscopic studies of developing mouse liver reveal sparse endoplasmic reticulum with morphologic development during the first week of life.

3. Bilirubin glucuronide formation is virtually absent in liver slices and homogenates obtained from human fetuses in late stages as well as from neonates who died of various causes within the first 3 days of life.

4. Phenobarbital and several other drugs increase liver UDPGT activity, enhance microsomal protein synthesis, cause proliferation of smooth endoplasmic reticulum membranes, and reduce serum unconjugated bilirubin concentrations in newborn infants.

5. Lifelong deficiency of UDPGT is associated with permanent nonhemolytic acholuric unconjugated hyperbilirubinemia in man and rats (Gunn strain).

The following summarizes evidence against this hypothesis (1):

1. UDPGT activity is relatively unstable and the enzyme has not been isolated, purified and characterized. The enzyme is a lipoprotein and specifically requires lecithin for activation after it has been delipidated (14).

Heterogeneity of UDPGT involved in N-glucuronide formation as compared with acyl and ethereal glucuronide formation seems well established. With regard to the latter, the situation remains confusing and multiplicity of UDPGT is suggested by varied effects of hypohysectomy and thyroidectomy, ions, pH and other factors on acyl and ethereal glucuronide formation. However, it remains uncertain as to whether these and kinetic differences with different substrates represent different enzymes or altered affinity for these substrates by a single UDPGT.

2. The pattern of development of UDPGT varies greatly with species and substrates. For example, UDPGT in newborn rat liver exceeds the enzyme activity found in adult rat liver (9). There has not been a systematic study performed in man or primate manifesting 'physiologic' jaundice using bilirubin as a substrate. Di Toro et al. studied the development of 4-methyl unbelliferone glucuronide formation in human liver biopsy specimens and observed little increase in UDPGT activity until the fourth week of life (6).

3. UDPGT activity levels may be misleading when interpreted physiologically (particularly after activation of the enzyme with detergents). For example, delayed development of hepatic UDPG dehydrogenase and UDPGT activities is observed in vitro in guinea pigs; however, studies based upon bilirubin infusion in vivo indicate that hepatic excretion of conjugated bilirubin is rate-limiting at all ages in the transfer of bilirubin from blood to bile in this species (10). Bilirubin glucuronide accumulates in the liver and plasma in guinea pigs less than 2 days old and hepatic excretory function does not attain adult levels until the third week of life. Guinea pigs do not manifest 'physiologic' jaundice... perhaps because their excretory limitation is physiologically more severe than the observed deficiency in UDPG dehydrogenase and UDPGT activities.

Dr. Lawrence Gartner at the Albert Einstein College of Medicine has pursued this problem by performing bilirubin infusion studies in Rhesus monkeys/1 hour to 18 days of age (11). These studies reveal the following important facts:

1. The newborn Rhesus monkey has 'physiologic' jaundice with rapid rise of serum unconjugated bilirubin concentrations from less than 1 mgm% at birth to 4.5 mgm% within 24 hours (phase I). A rapid decline to 2 mgm% occurs in the second day of life; the serum bilirubin concentration remains at this level for approximately 4 days (phase II) and then declines to 0.1 mgm% by the fifth day.

2. Mean endogenous excretion of bilirubin in bile rises during the first

48 hours of life to 500% greater than adult levels, and remains significantly elevated for the first 18 days of life.

3. Mean cumulative hepatic uptake of bilirubin is approximately 30% of adult values at birth and slowly reaches adult levels by the fifth day of life.

4. Hepatic UDPGT (bilirubin) activity is almost zero at birth but rises rapidly at 24 hours and attains adult levels by the third day of life.

5. Hepatic excretion of conjugated bilirubin becomes rate-limiting during the late newborn period as manifested by exceeding the biliary Tm for bilirubin with accumulation of conjugated bilirubin in the plasma and liver.

6. Mathematical analysis suggests that the hepatic conjugating ability during the first 24 hours of life is less than the load of bilirubin presented to the liver. If the bilirubin load (endogenous bile pigment production) were not 7 times that observed in adults, available UDPGT would be sufficient to prevent unconjugated hyperbilirubinemia. In stage II, relative deficiency in hepatic uptake is the major factor responsible for hyperbilirubinemia inasmuch as UDPGT activity is virtually normal. These studies suggest that 'physiologic' jaundice of the newborn Rhesus monkey and, probably human newborns, does not result from a single enzymatic defect in bilirubin conjugation but represents a complex interaction of many functional disabilities, each of which is probably subjected to various controls which are currently neither recognized nor appreciated.

A critical limitation in our knowledge concerns the mechanism whereby bilirubin is transferred from plasma into the liver. For at least four decades it has been known that following injection of 'physiologic' amounts of bilirubin and other organic anions, a large proportion of the injected dose is recovered within the liver within a matter of minutes. The mechanism responsible for this rapid and seemingly selective transfer from plasma into the liver remains unknown; however, several hypotheses have been studied experimentally:

1. Bilirubin is noncovalently bound to serum albumin and may enter the liver cell by pinocytosis as a pigment: albumin complex. This hypothesis is unlikely because when ^{131}I albumin and ^{14}C bilirubin were injected simultaneously into a rat, the bile pigment entered the liver at a rate many times faster than did albumin.

2. An active transport system may exist in the plasma membrane of the parenchymal liver cell. This is unlikely because hepatic uptake takes place despite inhibitors of energy metabolism, and 'throughput' studies by Goresky and others reveal bidirectional bilirubin flux consistent with a passive process.

3. Hepatic bilirubin uptake may be determined by hepatic blood flow and a high extraction ratio for bile pigment. Although hepatic blood must influence perfusion of hepatic lobules, definitive studies of this parameter have not been performed with respect to bilirubin. Up to 25% increase or decrease in hepatic arterial flow do not alter net hepatic uptake of bilirubin per unit time.

4. 'Unbound' bilirubin in plasma may be transferred across the plasma membrane of the liver cell by simple or facilitated diffusion. Net flux may be determined by either intracellular binding of bilirubin, subsequent metabolism or excretion, or by a plasma membrane carrier system. Kinetic 'throughput' studies by Goresky, Paumgartner and others indicate that the uptake process is saturable. This precludes the existence of simple passive non-carrier mediated diffusion as the mechanism of bilirubin entry into liver cells. If the transfer were simple passive diffusion, the uptake mechanism should not be saturable. These studies do not reveal the nature or site of the carrier mechanism. In theory, such facilitated diffusion could result from specific membrane molecules or pores, and/or cytoplasmic proteins having high affinity for bilirubin which enters the plasma membrane by virtue of its lipid solubility.

My colleagues and I have been studying the role of hepatic cytoplasmic proteins in facilitating the net flux of bilirubin and other organic anions from plasma into the liver. These studies have resulted in identification, purification and partial characterization of ligandin (Y protein) and Z, two organic anion binding proteins in liver, as well as studies regarding their function. Our hypothesis is that these proteins, particularly ligandin, influence the net uptake of organic anions into the liver specifically by regulating efflux from the cell into the plasma. It seems obvious that there is no way in which a substance in plasma can know what is in the liver and, therefore, influx must be independent of cytoplasmic binding proteins. By-products of these studies have been immunologic methods for quantitating ligandin and Z protein, immunofluorescent methods for studying cell localization, and detailed binding studies using circular dichroism and equilibrium dialysis.

In 1968, we determined that approximately 80% of intrahepatic bilirubin is associated with the 100,000 $\times$ g supernatant fraction of liver after radioactive bilirubin was injected into a rat (24). Fractionation by gel filtration revealed that radiobilirubin was found in two nonalbumin containing peaks called Y and Z. In subsequent years, a specific Y and a specific Z protein were purified and shown to account for at least 85% of the bilirubin binding in their respective fractions. Noncovalent binding of a large number of