LIVER UPDATE

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Proceedings of an
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symposium
on
liver physiology
and
disease

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Proceedings of an
International Symposium on Recent Advances in
Liver Physiology and Disease

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PREFACE

'Liver Update' was conceived in Chicago during the 1980 AASLD and IASL Meetings when our sponsor Dr Madaus & Company generously agreed to fund a small closed meeting in the Cape Town summer of 1981. At the conclusion of that meeting it was decided to repeat the conference every four years. Thus Liver Update 2 took place on the 25 - 27 February 1985. As before our aims were to bring together a group of enthusiastic experts, to place them in surroundings conducive to creative thought, to introduce them briefly to various topics by means of a State of the Art address and to provide ample time for the discussions which we hoped would follow.

No manuscripts were called for and the verbatum recordings have been condensed into this text by ourselves. In editing the proceedings we have tried to retain the enthusiasm as well as the very obvious expertise of the participants. Accolades belong to the delegates. We as editors accept the responsiblity for any deficiencies in the presentation.

R E K

J B K

G C

J T

Cape Town

March 1985

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Credit for ensuring that our 32 delegates from the USA, Europe, Asia, South America and Australia all arrived in good time must go to Mr Steve Allen of Miller Weedon Travel (Cape) and to our official airline South African Airways. We thank Mr R Melk and Mr C Miller for arranging an authentic South African evening at the Stellenbosch Farmers Winery.

The conference proceedings were recorded and captured on Wang Professional Computers. We thank Mr J Dunwoody of General Business Systems, Cape Town, for the loan of additional computers and for providing trouble-free equipment at all times. 'Liver Update' was printed and bound at the University of Cape Town. We thank Mrs C Bond and the staff of the Printing Department for their expertise, patience and tact.

We would like to thank members of staff of the Departments of Medicine and Surgery of the University of Cape Town, especially Mr V M Wells, Mr J Pasacovitch, Mr G Engelbrecht, Mr G Graham and Mr C Dryding for their assistance. To Professor J N Eloff of Kirstenbosch National Botanical Gardens go our special thanks. Mrs I Knight, B Russell, E Corrigali and C Woolgar assisted with the original typescript. Mrs V Stewart subedited the script. We thank Mr David Inglis of Bailliere Tindall W B Saunders for managing to publish this book so painlessly from a distance of 6000 miles. Our special thanks go to our Conference Organiser, Miss Carola Koblitz and to Mrs Isabel Batho who spent many hours converting our pencilled alterations into the completed text.

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CONTENTS

BILE PIGMENT METABOLISM, BILE FORMATION AND PORPHYRIA

CHAIRMAN: RALPH KIRSCH
STATE OF THE ART: TONY BASS

Bass: Since the topic of bilirubin metabolism and transport was discussed at *Liver Update I* four years ago, a sustained curiosity has resulted in continued advances in the understanding of bilirubin metabolism and transport. Historically, bilirubin awoke interest as the substance which produces the dramatic clinical sign of jaundice. Today, bilirubin stimulates the interest of hepatologists and biochemists by virtue of the fact that every advance in our understanding of its formation, transport and metabolism promotes greater understanding of the chemistry of organic compounds, of organic anion-protein interactions and of the transport and metabolism of drugs and other small molecules by the liver.

Today I will highlight some of the more recent advances in bilirubin metabolism, not a few of which have stimulated, and have in turn been stimulated by, controversy. The elucidation of the structure of bilirubin is of such fundamental importance that it is worth repeating. The old textbook depiction of bilirubin as a linear tetrapyrrole has been revised on the basis of nuclear magnetic resonance and radiographic diffraction studies.[1] These have shown that the naturally occurring bilirubin IX alpha isomer adopts an involuted 'ridge tile' structure as a result of hydrogen bonding between the propionic acid groups on one dipyrrole ring and the nitrogen and oxygen on the opposite pyrrole rings. This effectively shields the polar sites of bilirubin, rendering it virtually water-insoluble. Disruption of these internal bonds leads to the formation of water-soluble compounds and is achieved both by glucuronidation of the propionic acid groups and also by the formation of *cis-trans* isomers at the two outer methene bridges. In this latter process, which is promoted by the exposure of bilirubin to light, the natural Z,Z-isomer of bilirubin undergoes rotation at either one or both of the outer methene bridges to form E,Z, Z,E and E,E geometric isomers. This appears to be the major mechanism by which serum bilirubin levels are reduced in infants receiving phototherapy for neonatal jaundice.[2] The E isomers though water soluble are unstable. They form in the skin during light exposure and are rapidly transported from the skin to the plasma. These photo-isomers are transported to the liver bound to albumin and are taken up and excreted in the bile. Once in the bile they flip back once more to the water soluble Z,Z configuration. Bilirubin is transported in plasma bound to albumin. Binding occurs at a primary high affinity site[3] near lysine

residue 240, close to the single tryptophan residue, on the second domain of albumin. This site is distant from the primary fatty acid binding site on the third domain.[4] Albumin also possesses two lower affinity sites for bilirubin, but these are probably only occupied under conditions of extreme jaundice.

Several groups[4] have now reported the non-enzymatic formation of tight, probably covalent, binding of bilirubin to albumin under conditions of conjugated hyperbilirubinaemia. This newly recognized form of bilirubin-albumin interaction, variously termed biliprotein, bil-alb or delta fraction, forms between conjugated but not unconjugated bilirubin and serum albumin.[5] Weiss *et al.*[6] showed that during recovery from an episode of jaundice due to hepatobiliary disease bil-alb forms a progressively greater proportion of the total bilirubin fraction (up to 80%) with time. This finding explains two long-recognized and puzzling clinical phenomena. Firstly, the delayed resolution of jaundice long after resolution of active hepatobiliary disease. Resolution is prolonged since bil-alb is cleared at a slow rate reminiscent of the half-life of serum albumin although this has not been directly studied. Secondly, since bil-alb is not filtered by the glomerulus, the finding of this bilirubin protein complex explains the disappearance of bilirubinuria during convalescence at a time when the plasma conjugated bilirubin levels are still elevated. Several important questions are raised by these findings: Do other glucuronides form adducts with albumin? Do adducts occur with other binding proteins? And finally, does attachment of a ligand such as bilirubin modify the function and/or turnover of the proteins to which it is bound?

Bilirubin, bound to albumin arrives in the hepatic sinusoids where up to 50% is extracted by the liver. Weisiger *et al.*[7] proposed a model which has stimulated considerable thought regarding the mechanism of the uptake process. Their proposal of a hepatic albumin receptor provided a unique approach to resolving a paradox, which simply stated is as follows: small molecules such as bilirbin and fatty acids are transported through the liver tightly bound to albumin. The rates at which these small molecules disassociate from albumin have been studied and are simply too slow compared to the rapid transit time of albumin through the liver to account for the fraction of ligand which eventually remains behind in the liver.[8] Now there are both literal and broad interpretations of the albumin receptor model. The literal interpretation of the albumin receptor concept is that the ligand albumin complex associates with the hepatocyte plasma membrane either nonspecifically or via a specific receptor for albumin. The important point implied by this association is that it induces a conformational change in albumin effecting a more rapid or catalysed release of its bound ligand. This

interpretation was in part supported by isolated rat liver perfusion studies[8] which revealed a saturation kinetic behaviour of ligand uptake when increasing amounts of 1:1 molar ratio ligand: albumin were added to the perfusate.[7] Against this theory has been the failure to demonstrate a specific macromolecule fitting the role of an albumin receptor.[9] Indeed several other studies have suggested that a specific receptor does not exist. These include the demonstration by Stollman *et al.*[9] that the kinetics of the uptake of bilirubin bound to ligandin (an intracellular bilirubin-binding protein, which would not be expected to fit a specific receptor meant for albumin) closely resemble that of bilirubin bound to albumin.

There are broader interpretations of the albumin receptor concept, including recent work by Weisiger[10] in elasmobranchs. These studies have shown that the apparent saturation kinetics observed for the uptake of ligands from 1:1 molar ratio albumin:ligand complexes may be a result of a non-linear increase in the rate of reassociation of ligand with albumin as the concentration of the latter increases. The sum of dissociation and reassociation processes could thus limit the availability of free ligand so as to produce apparent saturation of uptake. This clearly provides a further important insight into the potentially limiting role of dissociation from albumin in the uptake process. It also provides an alternative explanation for some of the observed kinetics, which were thought to support the presence of an albumin receptor. If this is indeed the case, we are again left without an adequate explanation for the rapidity of the uptake process.

Some of the answers regarding bilirubin uptake may also lie in a specific hepatic sinusoidal membrane carrier for bilirubin.[11] Several membrane proteins with high affinity towards bilirubin and other organic anions have been isolated and suggested as possible receptor/carriers for these molecules. Indeed the evidence for carrier mediated uptake of bilirubin and other organic anions into the liver was reviewed at the last Liver Update meeting.

To date at least two membrane associated proteins have been described which might fit the role of bilirubin receptors and carriers. The first is a 55 000 dalton glycoprotein which may exist as a dimer of 100 000 daltons *in vivo*.[12,13] Immunohistochemical studies have shown that it is confined to the hepatocyte plasma membrane. The second is a 170 000 dalton protein which Tiribelli *et al.* have termed bilitranslocase.[14] This protein appears to have a trimeric structure made up of two alpha- and one beta-subunits.[14] Bilitranslocase has recently been shown to facilitate sulphobromopthalein (BSP) transport out of liposome vesicles down an electrochemical gradient.[15] The two organic anion-binding membrane proteins are distinct from two other similar membrane associated proteins, which appear to be

specific for fatty acids[16] and bile acids.[17] However the relationship between the two clearly distinctive bilirubin and BSP binding proteins is far from clear and further studies examining their role as carriers are needed.

Cytoplasmic binding proteins are next in line to receive bilirubin during its passage through the liver. Of these the ligandins, which bind organic anions and do double duty as the drug metabolizing glutathione transferases, have received considerable attention in recent years.[18] The role of these proteins in limiting the efflux of bilirubin following its uptake into the hepatocytes was elegantly demonstrated by workers at the Albert Einstein College of Medicine Liver Center several years ago.[19] However, their role in the intracellular transport of bilirubin remains unclear. Recently, it has been suggested that membrane-bound bilirubin is more rapidly utilized by microsomal glucuronyl transferase than is bilirubin bound to ligandin.[20] Indeed, a substantial portion of bilirubin may reach the microsomal enzyme by lateral diffusion through cellular membranes and by membrane-to-membrane transfer.[20] The abundant hepatic fatty acid binding protein, also known as Z-protein, appears to be predominantly, if not exclusively, involved with fatty acid transport.[21] It has recently been shown to behave differently from ligandin in that it does not appear to retard the efflux of bilirubin from liver.[22]

Of interest is the recent description by Kaplowitz's[23] group of an entirely new group of soluble cytoplasmic binding proteins, termed the Y' proteins, which they have resolved chromatographically from the ligandins. This heterogenous group of proteins includes Dv, a 35 000 dalton tetramer and D_1, a 40 000 molecular weight monomer. Both these proteins bind several organic anions with high affinity, with the notable exception of bilirubin, for which they have a low affinity. There are also two closely related 33 000 dalton bile-acid binding proteins present in this fraction. The fascination with binding proteins has thus continued, and the addition of these new members to the cast has added a new dimension of complexity to questions regarding the specific roles of these macromolecules in the transport and subsequent metabolism of bilirubin and small molecules.

We move now from the cytoplasm to the endoplasmic reticulum and the site of bilirubin conjugation with glucuronic acid by uridine diphosphate glucuronyl-transferase (UDPG-T). A long standing controversy based on the observed latency of UDPG-T and its stimulation by uridine diphosphate N-acetyl glucosamine (UDPNAG) is receiving renewed attention. The controversy centres on which of two models best explains the kinetic, induction and latency behaviour of the enzyme.[24,25] The compartmental model has the enzyme facing into the cisternal aspect of the endoplasmic reticulum and suggests that UDPNAG activates a permease

for uridine diphosphate glucuronic acid (UDPGA) in the endoplasmic reticulum membrane which allows it to gain access to UDPG-T. The constrained model proposes instead that the enzyme faces towards the cytoplasmic side of the endoplasmic reticulum and that its activity is highly modulated by its membrane lipid environment. In this model UDPNAG acts as an allosteric activator of the enzyme, promoting its binding of UDPGA. Both models have been elegantly defended. If the compartmental model should indeed prove to be correct, it would raise further questions about how unconjugated bilirubin enters and conjugated bilirubin exits from the cisternal space of the endoplasmic reticulum, or more explicitly, whether carriers for bilirubin exist on the endoplasmic reticulum membrane, as has been proposed for the plasma membrane.

There is little doubt that microsomal UDPG-T can sequentially catalyse the formation of bilirubin diglucuronide following the formation of monoglucuronide.[26,27]

An alternative mechanism of diglucuronide formation has been proposed by Roy-Chowdhury[28] in which the dismutation of two molecules of bilirubin monoglucuronide yields one molecule of diglucoronide and one molecule of unconjugated bilirubin, a process catalyzed by an enzyme present on the canalicular membrane. Seig *et al.*[29] have suggested that the apparent dismutation mechanism might be explained by a non-enzymatic artefact, i.e. dipyrrole exchange between two bilirubin monoglucuronide molecules. However, this may not be as compelling an explanation as it appears on the surface because of the fact that bilirubin is asymmetric around its central linking methyl bond. As a result of this a dipyrrole exchange will lead to the random formation of bilirubin III alpha and XIII alpha isomers in addition to the naturally occurring IX alpha isomer. Experimental studies have shown that not only the IX alpha isomer is formed, as might be expected, by the dismutation process.[28] However, the physiological significance of dismutation remains unclear for the time being and has not received much support.

The existence of several distinct molecular forms of microsomal UDPG-T has long been suspected on the basis of induction, kinetic and developmental studies as well as observations in the Gunn rat. Only recently has direct evidence for such molecular heterogeneity been presented. Roy-Chowdhury[30], using chromatofocusing, has separated eight isoforms of UDPG-T with subunit molecular weights in the region of 51 - 56 000 daltons.

On the basis of radiation inactivation analyses, it appears that the enzymes may exist as dimers of 110 000 daltons *in vivo*.[31] There is a fair amount of overlapping substrate specificity betweeen the different