Recent Advances in
HEPATOLOGY

EDITED BY

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E. ANTHONY JONES

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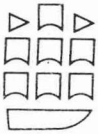

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Recent Advances in
HEPATOLOGY

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Preface

Many disciplines contribute to the growth of knowledge in hepatology and it is increasingly difficult for the practising physician and surgeon to follow the research literature. He relies more and more on the review articles scattered through the general medicine and specialist journals.

In the first and second volumes of this series we have selected chapter authors who are experts in a particular field of research but in addition have continued to work in clinical medicine. This approach has ensured that the chapters are palatable to the majority of clinicians as well as research workers. We hope we have saved the world's hepatologists hours of demanding reading.

London and
Bethesda, 1986

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E.A.J.

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1. Clinical evaluation of liver function

R. Preisig

INTRODUCTION

Clinical evaluation of liver function is usually based upon a complex interplay of information gathered from history, physical findings, laboratory tests, X-ray and/or ultrasound studies. The general aims of this integrated approach are fourfold, namely: (a) to establish the presence or absence of liver and/or biliary tract disease; (b) to provide a nosologic diagnosis; (c) to assess the severity of the disease; (d) to prognosticate evolution of the disease.

It cannot be overemphasized that a careful history, as well as a detailed search for and analysis of symptoms and signs are of utmost importance to clearly focused and cost-effective further investigations. Thus, recurrent episodes of scleral icterus following strenuous exercise or periods of fasting without major GI distress (suggesting Gilbert's syndrome) will evoke a different approach compared to a history of general malaise, lack of appetite, episodes of joint pains and upper abdominal discomfort (typical for hepatitis). Furthermore, the findings of a firm, enlarged liver together with palmar erythema and gynaecomastia establish the presence of (chronic) liver disease; as a consequence, the physician is directed towards the second, third and fourth of the above aims.

Evidently, a comprehensive review of all these aspects is not within the scope of this chapter. Rather, this article will concentrate on reviewing the clinically useful *biochemical tests* reflecting essential pathological features of liver disease, and the *clearance tests* for evaluation of liver function, pointing to recent developments in understanding and interpreting their results.

THE NORMAL LIVER

Setting a frame of reference for 'normal' liver function is fraught with several problems. Clearly, *liver weight*, being closely related to body size, is a primary determinant of *cell mass* and thus of liver function. Based on stereologic measurements, each additional gram of liver adds some 10^8 hepatocytes together with the necessary vascular and biliary infrastructure.[48] Correcting results of tests considered to measure 'functioning cell mass' for liver volume (as assessed by ultrasound techniques) or simply for body weight may be an important prerequisite for intersubject comparison.[13,19]

As an organ responsible for the homeostasis of an enormous variety of endogenous and exogenous compounds, function is not only based on tissue mass and structural integrity; it is also much dependent on the *supply of oxygen and nutrients* via the bloodstream and the regulatory effects of hormones. Not surprisingly, therefore, changes, for example, in $p0_2$ (high altitude) or in levels of growth hormone (acromegaly), may lead to functional alterations within the 'normal' liver.[38,41]

Additional variables affect hepatic function. Of particular importance may be inherited differences in certain hepatic enzyme systems. Despite well-established differences in the *capacity for acetylation*,[39] both fast and slow acetylators are considered variants within the normal, since consequences become evident only after administration of drugs. On the other hand, the recently discovered *defects in oxydative metabolism*[8,17,18,25] may present a different outlook. Although the 5–10% 'poor metabolizers' within a European population exhibit by all other criteria normal liver function, an up to 10 000-fold decrease in oxydative capacity has profound effects on the handling of exogenous compounds. In addition, preliminary evidence suggests that the disposition of endogenous substances (such as bile acids) may also be affected.

Exposure of the liver to a variety of *environmental chemicals* (insecticides, smoking, etc.) and other toxic compounds (ethanol) may have clear-cut effects on specific hepatic functions. Thus, the wide range of normal values for the clearances of antipyrine or caffeine[13,16,33,42] is in part based on the inducing effects of these pollutants. In addition, heavy drinking may lead to glutathione depletion (as reflected by plasma glutathione levels) in the absence of biochemical evidence of liver disease.[22] Finally, interactions of ethanol with hepatic drug metabolism — both inducing and suppressing — are well established.[23]

THE DISEASED LIVER

Essential pathologic features

The structural sequelae resulting from infections or toxic agents and from neoplastic infiltrates can be simplified to few essential components, such as: hepatocellular injury and necrosis, inflammatory reaction, cholestasis, fibrosis (± regeneration), vascular distortion.

Histopathologic study reveals that the most common liver disease may be seen as a relatively sterotype structural response, encompassing at least two of the above pathologic features. *Needle biopsy* of the liver (the 'gold standard') undoubtedly represents the most direct approach to establishing the presence or absence of liver disease. Interpretation of histopathologic findings may define or give valuable clues concerning the nosologic entity, particularly when (like in viral hepatitis) immunologic techniques are employed; except for extreme situations (e.g. severe necrosis), however, estimation of severity of the disease process within the entire organ is often difficult, and in view of potential sampling errors assessment of the remaining functional capacity is largely impossible. Albeit performed increasingly on an ambulatory basis, needle biopsy carried a definite — yet small — risk.

Commonly used liver tests

The essential pathologic features serve as a useful basis for understanding the clinically employed liver tests, which are based on serum levels of endogenous substances.

Hepatocellular injury (and necrosis) invariably leads to discharge of cell-contents into the bloodstream. Thus, the serum level of various enzymes (AST, ALT, etc.) and of iron and ferritin is increased. Since the kinetics of leakage and disposition from the body of these intracellular tracers are largely unknown, the extent of change in serum concentration is much influenced by the timing of blood collection in relation to the state of disease. Only following extensive cellular injury or chronic loss of functional mass is the synthetic capacity (e.g. prothrombin time) impaired.

The (non-specific) consequences of *inflammation* are best seen in the increase of acute phase proteins. Electrophoresis of serum protein exhibits an increase in the γ-globulin peak; with immunelectrophoresis, more characteristic changes (particularly in IgG and α_1-antitrypsin) are observed.

Assessment of *cholestasis* is usually made using the canalicular enzymes normally eliminated in bile. Obstruction to bile flow, be this intra- or extrahepatic, leads to retention (and in part increased synthesis) of hepatic alcaline phosphatase (AP), 5′-nucleotidase (5′NT) and gamma-glutamyltranspeptidase (GGT). Since GGT serum levels are also influenced by enzyme induction, AP and 5′NT may be preferable; the latter serves as a check that increased AP levels are not of gut or bone, but rather of hepatic origin.

Much effort has been devoted to find indicators of *fibrosis*. To data, with the availability of a commercial RIA, only the determination of type III procollagen peptide (PP) is making its way to the clinical laboratory. As has been shown in recent studies, PP levels in serum appear to reflect both fibrogenesis and extent of collagen deposit in the liver.[10]

Vascular distortion (resulting from fibrosis and/or regeneration) leads to impairment of blood perfusion. As a consequence, the development of intra- and extrahepatic shunting interferes with the (first-pass) extraction of bile acids reabsorbed from the gut. Although synthesis, storage, enterohepatic cycling and disposition of bile acids are influenced by additional variables, recent investigations suggest that the concentration of total, conjugated serum bile acids (measured with a RIA) in chronic liver disease may be interpreted as an expression of portal-systemic shunting.[26]

One of the most widely used tests, namely the measurement of serum bilirubin levels, serves to confirm the presence or absence of jaundice. Due to the complexities of bilirubin metabolism, increased serum levels cannot be assigned to a single pathologic feature; usually both cell injury and cholestasis are responsible for the derangement unless production is increased.[24] However, in subjects with Gilbert's syndrome (characterized by the lack of structural changes), determination of unconjugated bilirubin before and after an overnight fast or following administration of nicotinic acid[43] represents a reasonable and cost-effective approach to confirm the diagnosis.

Finally, the group of 'diagnostic tests', which are only indirectly related to deranged liver function, deserve mentioning. Test for hepatitis A and B markers, Epstein-Barr and cytomegalovirus infections have revolutionized the diagnosis of viral hepatitis. Antiactin (smooth muscle) and anti-DNA (antinuclear) antibodies (in titers of 1:50 or more) are helpful in the diagnosis of autoimmune hepatitis, antimitochondrial antibodies (in titers of 1:100 or more) a virtual prerequisite for the diagnosis of primary biliary cirrhosis. Similarly, determinations of ceruloplasmin, serum- and urinary copper, as well as ferritin and α_1-antitrypsin yield important clues to the presence of Wilson's disease, hemochromatosis and α_1-antitrypsin deficiency, respectively.

Interpretation of the commonly used liver tests

It must be kept in mind that the widely used liver tests represent single point measurements of a serum concentration (c). According to *pharmacokinetic principles*, c may be expressed as the ratio of dose (Q) over volume of distribution (VD), i.e. $c = Q/VD$.

At any point in time, the amount in the body equals the differences between Q_{in} (amount administered) and Q_{out} (amount eliminated), so that the above equation now becomes:

$$c = \frac{Q_{in} - Q_{out}}{VD}$$

Applying this principle to biochemical liver tests, Q_{in} may now be looked at as amount 'released' or amount 'synthesized', whereas Q_{out} implies disposition. Clearly, we lack the essential information on rate of synthesis, leakage or disposition of the different proteins measured; consequently, kinetic interpretation of these blood levels is not possible and estimation of severity of the disease hazardous.

Two further aspects need consideration. In a clinical setting, we do not usually screen for liver disease. Rather, our aim is *to confirm or negate a suspicion* (based on history, symptoms and signs) of hepatic disease, increasing the prevalence of liver disease markedly.[6] Further, liver tests are virtually always done in 'batteries', thus minimizing the false negative results. Based on these premises *predictive accuracy* (i.e. the probability that a positive test indicates the presence of hepatic disease) of two or more positive results is very high, being of the order of 95% or better.[11] Indeed, using a combination of tests such as AST, AP, GGT, conjugated serum bile acids (and possibly immunelectrophoresis) makes it highly unlikely that a subject with significant liver disease is missed.

With the exception of the above mentioned 'diagnostic tests', conventional liver tests provide little help in *diagnostic discrimination*.[24] This is not surprising in view of the fact that the common liver diseases are characterized by a mixture of 'essential pathologic features' leading to significant abnormalities of several or all conventional tests.

Although *monitoring of liver disease* with conventional tests is daily routine, little effort has been made to define optimal timing and to suggest a cost-effective approach. Nevertheless, in acute liver disease, return of all values to normal usually signifies improvement or even healing. In this sense, the conventional tests are of prognostic help.

The clearance tests: general aspects

Pharmacokinetic study of the fate of exogenous compounds handled principally or exclusively by the liver permits functional dissection of the liver in quantitative terms. The underlying concept is as follows:

$$\text{Clearance} = \frac{\text{hepatic removal}}{\text{arterial plasma conc.}}$$

Hepatic removal = plasma flow (arterial − hepatic venous conc.)

$$\text{Clearance} = \text{plasma flow}\left(\frac{\text{arterial} - \text{hepatic venous}}{\text{arterial}}\right)\text{conc.}$$

The term in parenthesis $\left(\frac{\text{a-hv}}{\text{a}}\right)$ is the hepatic extraction ratio (E).

Depending upon the choice of tracer (i.e. its hepatic extraction ratio), clearance is either *flow- or capacity-limited*. As is evident from the above equation, clearance of a flow-limited tracer (e.g. indocyanine green, lidocain, nitroglycerine) equals actual

hepatic plasma flow only to the extent that E approaches unity. Whereas in the normal liver E of these tracers is around 70–90%, in subjects with liver disease it may decrease to <30%.[35] In such patients, therefore, clearance values underestimate hepatic plasma flow quite markedly. The disposal is then largely determined by the cell mass. Such problems are avoided with capacity limited compounds (aminopyrine, antipyrine, caffeine), whose E in liver normal subjects is <25%, and whose protein binding is very low or even negligible. By choosing a large dose (0.5 g/kg body weight), which saturates the enzyme system responsible for its elimination, galactose is 'turned into' a capacity limited substrate. Unfortunately, with this dose urinary clearance now accounts for approximately 10–20% of the amount injected; in addition, galactose is also removed into an ill-defined extrahepatic compartment (see Table 1.1).

Table 1.1 Sampling sites for clearance tests of hepatic function

Sampling sites	Blood	Breath	Saliva	Urine
Test compound used	Indocyanine green Galactose Caffeine Antipyrine	Aminopyrine Coffeine Galactose	Caffeine Antipyrine	Debrisoquine Mephenytoin Dextromethorphan

A variant of this clearance approach is used to assess *acetylation* (sulfadimidine) or *hydroxylation* (debrisoquine, dextromethrophan, mephenytoin) of a given compound. Rather than calculating (renal) clearance, the ratio of excretion of the parent compound versus the major metabolite is obtained (so-called metabolic ratio). This procedure, chosen for practical reasons, is sufficient for delineating poor and efficient metabolizers.[17,25]

Finally, the pharmacodynamic response after i.v. and p.o. administration of glyceryl trinitrate has been measured using finger-plethysmography to quantitate first-pass elimination and *shunting*.[37]

As shown in Table 1.1, collection of samples for analysis may be performed from blood, breath, saliva or urine. Whenever possible, the least invasive approach is used. Our present choice is to measure indocyanine green and galactose in plasma, $^{14}CO_2$ derived from aminopyrine in breath, caffeine in saliva and the pharmacogenetic tracers in urine.

Individual clearance procedures

Flow-limited tests

Indocyanine green (ICG), which has been used extensively for measurement of hepatic blood flow using the infusion and extraction technique, is considered the indicator of choice. This dye is taken up exclusively by the liver and eliminated in the bile virtually unchanged.[34] The normal liver extracts the dye with an efficiency of 70–90%.[35] Using a bolus injection of 0.5 mg/kg body weight, venous blood samples are collected at regular intervals in the opposite arm during 20 minutes. When plotted on semilogarithmic paper, the plasma concentration-time curve yields a straight line. VD is then obtained as ratio of dose to the extrapolated C_0, the elimination rate constant k as ratio of ln2 to the graphically read half-life. Clearance is calculated as the product of $k \times VD$.

Since the ICG-E in a given patient with liver disease remains unknown (unless

hepatic vein catheterization is performed), the clearance obtained cannot be directly related to values of hepatic plasma flow. For practical purposes it is therefore sufficient to obtain k. In subjects without liver disease, k ranges from 0.14 to 0.28 min^{-1}.

Although methods for measuring the fraction of portal blood bypassing hepatocytes have been described previously,[31,32] they are invasive requiring catheterization of the portal vein. In our department, Porchet and Bircher have designed an approach using the bioavailability principle applied to *nitroglycerin* (glyceryl trinitrate).[37] When swallowed in therapeutic doses (0.6 mg), liver normal subjects show virtually complete first-pass elimination and hepatic inactivation of nitroglycerin; therefore a vascular effect — assessed by digital plethysmography — is not measurable. Patients with liver disease leading to porta-systemic shunting and decreased extraction given the same dose now exhibit changes in their pulse waves, attesting to increased systemic availability of the drug. The extent of these changes can be quantified on the basis of an intravenous dose-response curve (i.v. infusion of nitroglycerin between 2 and 20 μg/min). In view of the short plasma half-life of nitroglycerin, the procedure takes about 2 hours. Recent work (Fig. 1.1) has supported the principle of the procedure

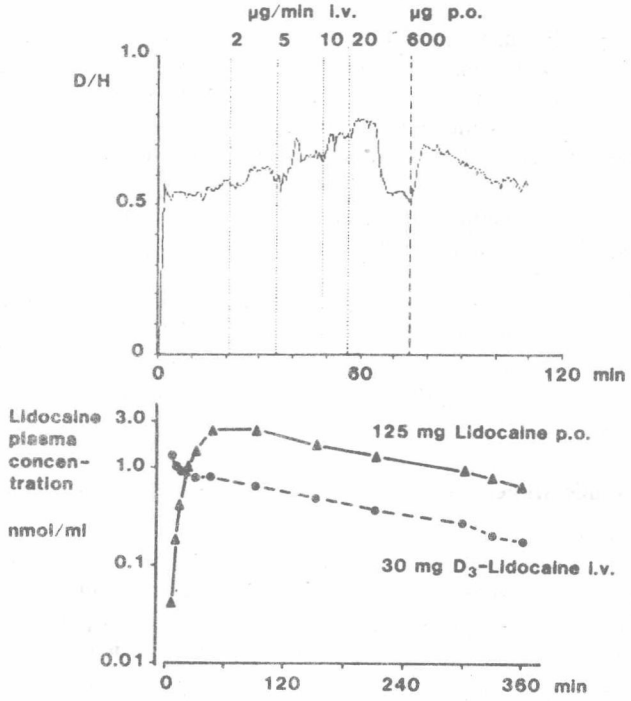

Fig. 1.1 Estimation of portosystemic shunting in a 55-year-old male patient with biopsy documented alcoholic cirrhosis and splenomegaly using computerized digital plethysmography (original tracing, *upper panel*). Successive i.v. dosing of 2, 5, 10 and 20 μg/min of glyceryl trinitrate evokes an increasing response (expressed by ratio pulse wave/notch height D/H). Based on this dose-response curve, systemic availability of the subsequent p.o. dose is 40%. In the same patient, systemic availability assessed with simultaneous p.o. and i.v. administration of lidocaine (*lower panel*) is 70% (calculated from $^{AUC}p.o./AUC_{i.v.} \times {}^{dose}i.v./dose_{p.o.}$). Presumably, the difference is due to prehepatic metabolism of glyceryl trinitrate.

comparing the bioavailabilities of notroglycerin and lidocaine.[27] Based on these studies it should be possible to correct for prehepatic nitroglycerin inactivation thus obtaining 'true' shunt flow.

Capacity-limited tests

Given in an i.v. dose of 0.5 g/kg body weight, *galactose* disappears from the bloodstream in a zero order fashion.[3,12,40] With this procedure, the rate-limiting metabolic step in hepatic elimination (phosphorylation by galactokinase) is saturated. Plasma concentration (measured photometrically or fluorimetrically) in samples collected at regular intervals between 20 and 50 minutes after injection yield a straight line on a linear scale. Using the dose administered, *galactose elimination capacity* (GEC) is calculated according to standard procedures.[3] The normal range for GEC (6–9 mg/min/kg) obtained in this way requires further correction, since approximately 2.5 mg/kg/min galactose are processed by extrahepatic, non-renal mechanisms.[3] The resulting 3.5–6.5 mg/kg/min may be regarded as equivalent to the 'functioning cell mass'. The procedure is safe, since the injected sugar may be considered innocuous.

Using uniformly (^{14}C) or (^{13}C) labelled D-galactose, it is possible to perform a *galactose breath test*.[46] Following modification of the method, we were able to demonstrate that the slope of the $^{14}CO_2$ specific activity in breath (obtained during 45 minutes) was highly correlated ($r = 0.87$, $n = 26$) with GEC.[12] Little is known about the kinetics of CO_2 formation. Since pyruvate is probably the end product of galactose metabolism yielding acetate and CO_2, the galactose breath test might be useful to study the fate of C_1-fragments in the organism.

In the 12 years since its original description,[20] the *aminopyrine breath test* has gained wide acceptance. The characteristics of the test compound, namely distribution in body water, negligible binding proteins, low (<20%) hepatic extraction, metabolism mainly by the liver with cleavage of the label being the rate-limiting step, make it a close to ideal substrate for measuring hepatic monooxygenase activity by analysis of exhaled, labelled CO_2.[2] Originally, pharmacological doses of the cold compound together with 7.4×10^4 Bq of (^{14}C-dimethyl) aminopyrine were administered p.o. and breath samples collected up to 8 hours; it was possible to demonstrate that elimination of the parent compound from plasma is closely correlated with $^{14}CO_2$ disappearance from breath.[2,4,36] Subsequently, the procedure was simplified to a single i.v. application of a tracer dose (7.4×10^4 Bq) of aminopyrine, followed by collection of breath samples (e.g. at 15-minute intervals) up to one hour.[36] If done on an out-patient basis, care must be taken to keep the patient at rest, in order to achieve basal endogenous CO_2 formation. As we have shown recently (Fig. 1.2), the pattern of the curve obtained during the entire period of 60 minutes may give additional clues, such as the suspicion of hepatic enzyme induction. Clearly, the test is simple to perform, practically non-invasive and the estimated absorbed radiation dose (approximately 5 mrem) considerably smaller than many diagnostic procedures used in radiology or nuclear medicine. Agranulocytosis has not been reported after the use of single doses of aminopyrine.

Following elucidation of its major routes of metabolism in man,[1] *caffeine* appeared as a virtually ideal 'model compound' for measureing liver function. In the doses needed, it is completely innocuous; in addition, it shares most of the characteristics (distribution in body water, low protein binding, low extraction and practically complete hepatic metabolism by oxidative demethylation) of aminopyrine. Therefore,

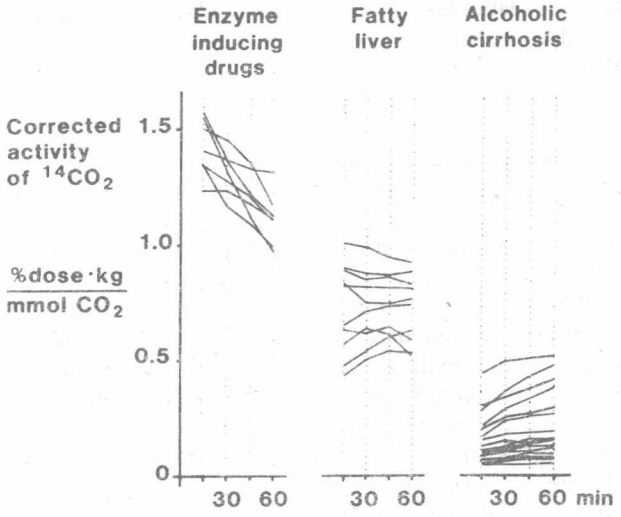

Fig. 1.2 Corrected activity of $^{14}C0_2$ in four breath samples 15, 30, 45 and 60 minutes following i.v. injection of a tracer dose (7.4×10^4 Bq) of (^{14}C-dimethyl) aminopyrine. The subjects on enzyme inducing drugs were epileptics taking phenytoin and/or phenobarbital; fatty livers and cirrhosis were biopsy-documented. In addition to absolute values of $^{14}CO_2$ breath activities at each time point, which are clearly different in the three groups, the pattern of the entire curve may yield additional diagnostic clues.

its use as a substrate for a *caffeine breath test* (CBT) was investigated. Animal experiments and studies in man[49] confirmed the feasibility of a CBT, demonstrated that demethylation largely reflects hepatic cytochrome P_{1-450} activity and that the dominant pathway was demethylation of the 3-position. This was confirmed in a larger group of normal subjects.[16] The CBT was then explored in 26 subjects with liver disease and the results compared to 10 normal volunteers.[42] These studies showed a close linear relationship between activity of $^{14}C0_2$ in breath and plasma clearance over a wide range of clearance values ($r = 0.83$, $n = 36$). The CBT may be viewed as a powerful tool to quantify hepatic functional impairment.

The future of caffeine as a compound for measuring liver function may be even more promising in the form of a *saliva test*, thus avoiding administration of radioactivity. This approach is based on the close and constant relationship of saliva/serum concentrations of caffeine (ratio: 0.74). Following p.o. dosing (280 mg caffeine added to decaffeinated coffee) between 2 and 4 p.m. the preceeding day, an overnight clearance is calculated from two saliva samples obtained at bedtime and upon arising the following morning.[15] As shown in Figure 1.3 this test yields data comparable to the CBT. Normal clearance values were $1.7 \pm$ SD 0.54 ml/min/kg, showing no overlap with those in biopsy-confirmed cirrhotics (0.60 ± 0.36). Presumably, the spectrum of functional change in liver disease may thus be quantified using a completely non-invasive and safe procedure. The time-honoured *antipyrine* has come into focus again following description of a method to measure its clearance from a single sample of saliva.[7] This compound undergoes biotransformation mediated by different cytochrome P-450 enzymes. Due to virtual identity of saliva and plasma concentrations, clearance can be calculated from a saliva sample collected approximately 24 hours after dosing (15 mg/kg body weight p.o.) assuming a fixed VD of 40 litres. It is