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The Aging Liver

Drug Clearance and an Oxygen Diffusion Barrier Hypothesis

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Summary

A change in drug clearance with age is considered an important factor in determining the high prevalence of adverse drug reactions associated with prescribing medications for the elderly. Despite this, no general principles have been available to guide drug administration in the elderly, although a substantial body of clearance and metabolism data has been generated in humans and experimental animals. A review of age-related change in drug clearances established that patterns of change are not simply explained in terms of hepatic blood flow, hepatic mass and protein binding changes. In particular, the maintained clearance of drugs subject to conjugation processes while oxygen-dependent metabolism declines, and all *in vitro* tests of enzyme function have been normal, requires new explanations.

Reduction in hepatic oxygen diffusion as part of a general change in hepatocyte surface membrane permeability and conformation does provide one explanation for the paradoxical patterns of drug metabolism, and increased hepatocyte volume would also modify oxygen diffusion path lengths (the 'oxygen diffusion barrier' hypothesis). The reduction in clearances of high extraction drugs does correlate with observed reduction in hepatic perfusion.

Dosage guidelines emerge from these considerations. The dosage of high clearance drugs should be reduced by approximately 40% in the elderly while the dosage of low clearance drugs should be reduced by approximately 30%, unless the compound is principally subject to conjugation mechanisms. If the hepatocyte diffusion barrier hypothesis is substantiated, this concept may lead to therapeutic (preventative and/or restorative) approaches to increased hepatocyte oxygenation in the elderly. This may lead to approaches for modification of the aging process in the liver.

Prescribing drugs for elderly people is a major professional challenge for many medical practitioners.[1,2] On one hand, the incidence of most illnesses increases with age; hence, the elderly are likely to benefit from the use of appropriate medications. On the other hand, the elderly more frequently suffer from the adverse effects of drugs. Hurwitz,[3] in a survey of 1268 patients admitted to a general hospital, found that the rate of adverse reactions more than tripled in patients over 70 years old. This trend has also been observed in general practice,[4] hospital outpatient departments[5] and possibly to an even greater extent amongst elderly in nursing homes.[6] It has been argued that old age itself is not an independent risk factor for adverse drug reactions but merely a marker for comorbidity, altered pharmacokinetics and polypharmacy, however, this argument was advanced in an attempt to prevent exclusion of older volunteers from clinical trials.[7]

The need to reduce adverse drug reactions has stimulated much research into the effects of age on drug metabolism. It is surprising that despite significant research efforts, the effect of age of hepatic drug metabolism continues to be a controversial issue.[1,8-10] The observed changes in the clearance of drugs that undergo hepatic metabolism were originally attributed to changes in hepatic enzyme activity[11] and more recently to altered hepatic size and blood flow.[8-10,12-15] However, it has been recognised that none of these invoked mechanisms fully explain the age-related changes in hepatic drug clearance seen *in vivo*.[8,9]

In this paper we present a review of the effects of age on those general aspects of liver function and activity that could impact on drug metabolism and also review the adequacy of current pharmacokinetic theories to explain the effects of age on hepatic drug clearance. This paper focuses on systemic drug clearance because of the inconsistencies in the data available for presystemic clearance and aging.[16] As a result of these reviews, we propose alternative theories which may help explain some of the observed, but unexplained, effects of age on hepatic drug metabolism. If validated, these proposals provide a practical clinical basis for safer prescription choices and drug design as well as dosage guidelines in the elderly.

1. Aging and the Liver

1.1 Morphological Changes

Macroscopically the liver is described as undergoing 'brown atrophy' with old age.[8,15] Initially, aging was found to be associated with a 20% reduction in liver weight in males and an 11% reduction in females.[17] This trend has been confirmed many times in both humans and animals using a

variety of techniques. In general the reduction of liver size is noted to be in the order of 25 to 35% (table I).[17-27] The brown appearance is secondary to the accumulation of pigmented waste products within hepatocytes. The major pigment is the 'wear and tear' substance, lipofuscin, which consists of the end products of lipid peroxidation in lysosomes.[19]

Microscopic changes which occur in the liver with age have not been clarified fully. It appears that there is a reduced number of swollen hepatocytes. Electron microscopic studies of aging rats have yielded variable reports of hepatocyte volume, increasing by 4,[28] 13 to 18[29] and 25%.[30] Other changes include reduced endoplasmic reticulum and increased nuclear polyploidy and binucleation.[30,31] The extracellular and vascular spaces also appear to increase.[27,30] However, these findings have not been confirmed by all groups.[31] Mitochondria become swollen but the number of mitochondria per hepatocyte does not change.[19,32] Overall, any age-related microscopic changes are subtle and Schmucker[29] concluded that 'hepatic fine structure is markedly similar in young and old'.

1.2 Physiological Changes

The main age-related change in the physiology of the liver is a substantial reduction in blood flow of about 40%. This has been documented in humans and rodents, using a variety of techniques including dye clearance, indicator distribution and portal Doppler-ultrasound (table II).[23-26,33-35] Hepatic arterial supply as a proportion of cardiac output is constant[24] and there are no major changes in portal venous haemodynamics[36] with age. This suggests that the reduction of blood flow is caused by diminished splanchnic blood flow which reduces input of blood into the portal vein with some compensatory increase of hepatic arterial supply, possibly secondary to activation of the hepatic artery buffer response.[37] Bile flow and bile salt formation are reduced by about 50% reflecting, at least in part, impairment of energy-dependent and microtubule-dependent transport processes.[38]

Table I. Percentage reduction of hepatic size (as a proportion of bodyweight) with age

Age-related reduction in hepatic size (%)	Reference
Humans	
20 (M), 11 (F)	17
36	18
35	19
17	20
25	21
29	22
28 (M), 44 (F)	23
Rats	
30	24
18	25
29	29
30	27

1.3 Biochemical Changes

The liver has a pivotal role in the regulation of the metabolism of carbohydrates, proteins and lipids. The effect of aging is variable with one older review noting that aging was associated with no change of enzyme activity in 80% of reports, increased activity in 13% of reports and decreased activity in 7% of reports.[39] The synthesis of proteins, lipid and glucose decreases with age.[31,32,40] Total protein synthesis is reduced by about 50% in aging rodent livers and this may be associated with impaired hepatic degradation of proteins.[40] Greenblatt[41] in a study of 11 090 individuals found that serum albumin decreases from 39.7 g/L to 35.8 g/L with age. More recently it was reported that serum albumin decreases by 0.54 g/L per decade.[42] It is surprising, therefore, that specific biochemical studies in aged rats have tended to show an increase of albumin synthesis.[43]

Antioxidant activity in the liver appears to be reduced with age. There are age-related declines in the activity of hepatic superoxide dismutase[44] and glutathione peroxidase.[45] However, it is something of a paradox that chemical-induced hepatotoxicity is unchanged or even reduced in aged rats[46,47] and the livers from aged rats are more resistant to reoxygenation injury.[48]

Table II. Influence of age on hepatic blood flow

Age-related reduction in blood flow (%)	Reference
Humans	
1.5% p.a.	33
53	23
20	34
49	35
Rats	
55	24
49	25
35	26

1.4 Clinical Implications

There are no major recognised age-specific liver diseases, but the prognoses for many liver diseases tend to be worse in the elderly presumptively secondary to delayed presentation and comorbidity.[49] Routine clinical tests of liver function do not change significantly[50] although albumin concentrations decrease slightly.[41,42] Ischaemic hepatitis does occur more frequently in the elderly, but this may reflect the age-related increase in degenerative cardiovascular disease.[51]

The major clinical implication of hepatic aging is usually considered to be pharmacological.[8,49] The hepatic elimination of many medications and other xenobiotics is impaired with age. This may contribute to the higher prevalence of adverse drug reactions in the aged and lead to an increased exposure of the elderly to potential disease-inducing agents.

1.5 Drug Metabolising Enzymes

In 1964 Kato et al.[52] reported an age-related reduction of phase I, mixed function oxidase activity in rats. This result was confirmed in several strains of male rat using microsomal preparations for cytochrome P450, cytochrome b_5, and reduced nicotinamide adenine dinocleotide phosphate (NADPH) cytochrome c reductase activity. *In vitro* studies of individual enzymes such as hexobarbital hydroxylase, aminopyrine N-demethylase, ethylmorphine N-demethylase and 7-ethoxycoumarin *O*-deethylase confirmed an age-related reduction of the activity of phase I enzymes in male rats, but the effect was less in female rats (reviewed by Van Bezooijen[31]). These trends were supported by *in vivo* studies of the clearance in rats of drugs that undergo phase I metabolism, such as propranolol.[53] The fact that the decline in drug metabolism was gender-specific led to the conclusion that any age-related effects on phase I metabolism are related to hormonal changes specific to the male rat and not to the aging process itself.

Subsequently, crucial studies were performed on microsomal enzyme preparations from human livers. It was found that aging was not associated with any change in hepatic microsomal protein content nor in the activities of NADPH cytochrome reductase, aldrin epoxidation, 7-ethoxycoumarin *O*-de-ethylation, epoxide hydrolase and aspirin (acetylsalicylic acid) esterase.[14,54] No relationship has been found between age and the activities and content of various CYP enzymes and mono-oxygenases determined from microsomal preparations from liver resection specimens.[55-57] However, all of these experiments have been performed *in vitro* and any effects of age on the *milieu interieur* of the hepatocyte or on solute transport would not be detected.

Table III. Influence of age on the clearance of some drugs that are metabolised by the liver[59]

Reduced clearance	No change
Amitryptiline	Caffeine
Antipyrine	Diazepam
Chlormethiazole	Digitoxin
Diltiazem	Isoniazid
Fentanyl	Oxazepam
Ibuprofen	Paracetamol (acetaminophen)
Imipramine	Phenytoin
Indocyanine green	Salicylic acid
Lignocaine	Temazepam
Morphine	Valproic acid (valproate sodium)
Naproxen	Warfarin
Nifedipine	
Pethidine	
Propranolol	
Theophylline	
Verapamil	

Table IV. Terms describing the metabolism of drugs by the liver and their physiological and/or biochemical interpretation

Term	Pharmacological meaning	Interpretation
Phase I	Structural alteration by oxidation, reduction, hydrolysis	Activity of specific microsomal enzymes
Phase II	Conjugation with chemical groups such as glucuronide, sulphate, acetate, glutathione	Activity of specific cytosolic enzymes
Capacity-limited	Low hepatic extraction (less than 0.3)	Liver size, total enzyme activity, protein binding
Flow-limited	High hepatic extraction (more than 0.7)	Liver blood flow

There does not appear to be a significant reduction of phase II metabolism with age. Most *in vitro* experiments in male rats have found that the activities of glutathione transferase and UDP glucuronyltransferase are unchanged in old age.[31] Paracetamol (acetaminophen) glucuronidation and sulphation does not change with age in human liver preparations.[58] Possible extrahepatic changes in conjunction have not been defined.

1.6 Drug Clearance *In Vivo*

Most studies of aging and drug metabolism have involved simple pharmacokinetic investigations that report the elimination of a single drug *in vivo*. Results indicate a superficially confusing spectrum of drugs with reduced clearance and others with unchanged clearance (table III).[49,59,60] In order to find any trends amongst this data, it is necessary to apply some of the basic principles of hepatic drug clearance.

2. Principles of Hepatic Drug Clearance

By examining the structure and behaviour of some drugs and their metabolites it is possible to infer certain characteristics about the aging liver.[60] Table IV summarises the different terms used to describe hepatic drug metabolism and the physiological and biochemical interpretation of such terms.

Hepatic clearance (CL_H) is defined by the simple equation:

$$CL_H = Q \times E \qquad \text{(Eq. 1)}$$

where Q is the hepatic blood flow and E is the hepatic extraction fraction, the fractional removal of drugs by the liver. Some substances eliminated by the liver have an extraction that approximates unity. This type of metabolism is called 'flow-limited' because the hepatic clearance will be almost equal to hepatic blood flow. Using this principle, the clearances of highly extracted dyes, such as indocyanine green and bromosulphthalein, have been used clinically to estimate hepatic blood flow.[33,35]

On the other hand, the clearance of drugs with a low extraction fraction is not influenced by blood flow. The metabolism of these drugs is influenced by intrinsic clearance (a term that describes total enzyme activity and liver mass) and/or protein binding and is termed 'capacity-limited'. The clearances of compounds such as antipyrine and galactose have been used to determine liver size.[21]

The relationship between hepatic clearance (CL_H) and parameters such as hepatic flow (Q), intrinsic clearance (CL_{int}) and the unbound fraction (f_u) has been described by various models. The simplest model is the venous equilibrium model[61] which assumes complete mixing of substrates within the liver and can be summarised thus:

$$CL_H = \frac{Q \times f_u \times CL_{int}}{Q + f_u \times CL_{int}} \qquad \text{(Eq. 2)}$$

Phase I and phase II are terms used to describe the major enzymatic pathways in the liver that metabolise drugs and other xenobiotics. Both pathways tend to increase the water solubility of compounds and facilitates renal excretion. Phase I reactions alter the structure of a compound by oxidation, reduction, hydrolysis and demethylation and are performed mostly by the CYP system of enzymes within the endoplasmic reticulum. Phase II reactions involve the addition of polar chemical groups such as glucuronide, sulphate, glycine,

Table V. Effects on drug clearance and enzyme activity that are predicted by models of altered hepatic drug metabolism

Model	Drug clearance *in vivo*	Enzyme activity *in vitro*
Physiological models		
Reduced blood flow (↓Q)	↓ F-L	↔
Reduced intrinsic clearance (↓CL_{int})	↓ C-L	↓ or ↔
Reduced protein binding (↑f_u)	↓ highly protein bound and C-L	↔
Disease-based models		
Intact hepatocyte	↓ F-L and C-L (proportional)	↔
Sick cell	↓ C-L (proportional)	↓
Impaired uptake	↓ highly protein bound and hydrophilic	↔
Oxygen limitation	↓ phase I	↔

Abbreviations and symbols: C-L = capacity-limited; F-L = flow-limited; ↓ = decreased; ↑ = increased; ↔ = unchanged.

glutathione and acetate. These reactions occur mainly in the cytosol. Recent studies indicate that the activity of phase I enzymes is more dependent on the delivery of oxygen than phase II enzymes.[62-64] Many drugs, including some tricyclic antidepressants and benzodiazepines, are metabolised extensively by both pathways prior to excretion in the urine or bile.

Drug elimination can also be influenced by many other factors such as drug absorption, extrahepatic metabolism, tissue distribution, protein binding and renal excretion. Therefore, in order to assess the effects of age on the liver, it is preferable to examine drugs that are not influenced by renal excretion or other routes of elimination and where absorption is not affected by age.

It should be noted that these traditional approaches to the understanding of hepatic drug metabolism make the assumptions that there are no limitations on the supply of oxygen and/or other cofactors, and that other substrates including drugs have unimpeded access to enzymes.[65,66] It also assumes that any changes in physicochemical parameters such as intracellular pH and osmolality will not impact on enzyme function.

3. Physiological Theories of Aging and Hepatic Drug Metabolism

There is considerable variability in the results of clearance studies in the elderly, reflecting confounding factors such as differences between the elderly and very elderly, frailty,[13,67] comorbidity, polypharmacy, smoking and alcohol intake, altered nutrition[60,68,69] and enzyme induction.[10] Nevertheless, it should be possible to determine whether the effects of age on drug metabolism is secondary to age-related changes in blood flow (Q), protein binding (f_u) or enzyme activity and liver size (CL_{int}). Table V summarises the effects on clearance and *in vitro* activity that would be predicted if there were major changes in each of these parameters.

3.1 Hepatic Blood Flow

Old age is unambiguously associated with a reduction in hepatic blood flow of about 40% (section 1.2). It is important to note:

- that the measurement of blood flow has been determined using non-clearance methods such as ultrasound[34] and indicator distribution[24]
- when indocyanine green clearance has been used to measure blood flow, it has been confirmed that hepatic extraction is not influenced by age in the rat.[26]

These experimental issues are important because they overcome the circular argument that the clearance of drugs is reduced because hepatic blood flow, when measured using drug clearance techniques, is reduced.

In 1984 Woodhouse et al.[14] reported that there was no relationship between age and the activity of various oxidative enzymes in human liver microsomal preparations. On the basis of this study and

several similar studies, researchers from the Newcastle-upon-Tyne group suggested that the effects of aging on hepatic drug metabolism must be secondary to reduction of blood flow and liver size.[9,10,12-15] Any reduction in hepatic blood flow would only be expected to be associated with a concomitant reduction in the clearance of drugs with a high extraction fraction (eq. 1).

A detailed review by Durnas et al.[59] listed approximately 200 human studies of the effects of aging on 100 different drugs that are metabolised by the liver. From this review we have summarised the effects of age on the metabolism of those drugs that are usually considered to undergo flow-limited metabolism as well as capacity-limited, phase I and II metabolism (table VI). It can be seen that there is a consistent effect of age on the clearances of flow-limited drugs, most of which are reduced by about 30 to 40%, correlating well with the age-related reduction in blood flow (table II). Clearly, blood flow has a major influence on drug metabolism in elderly people and the dosage of any highly extracted drug should be reduced by nearly half for this reason. However, the reduction in hepatic blood flow does not explain all age-related changes, for example the reduction in the clearances of some capacity-limited and phase I drugs (table VI).

3.2 Intrinsic Clearance

Intrinsic clearance is a term used to describe total hepatic drug metabolising enzymes. It is influenced by changes in liver size, enzyme mass or enzyme activity. Measurement of liver weight and volume has confirmed that old age is associated with a reduction in liver size (table I). This logically would be expected to be associated with a reduction in the clearance of capacity-limited drugs (section 2). However, it can be seen from table VI that there is no obvious relationship between age and the clearance of capacity-limited drugs. The clearances of some drugs are not affected by age, but many of these drugs may be influenced by protein binding (e.g. warfarin). It is conceivable that the age-related reduction in albumin and associated increase in the unbound fraction of these drugs could compensate for a possible reduction in hepatic metabolism.

Some studies indicate that even when there is a reduction in the clearance of a capacity-limited drug, this does not always correlate with the reduction in liver size. Bach et al.[20] reported that the clearance of both antipyrine and free phenytoin was reduced in elderly people even after correction for liver size as determined by ultrasound. Recently, Sotaniemi et al.[70] studied the effects of aging in 226 individuals. They found that there was a 29% reduction of antipyrine clearance and a 32% reduction of liver CYP content measured from liver biopsy specimens. However, the reduction in cytochrome P450 content occurred several decades before any reduction in drug clearance was observed.

Reduced intrinsic clearance could also occur as a result of altered enzyme activity. The experiments of Woodhouse and associates[14,54] appear to have excluded this possibility in humans (section 1.5).

In summary, there are no age-related changes of *in vitro* enzyme activity and there is a poor relationship between the clearance of capacity-limited drugs and age, and some studies have failed to find a close association between liver size and drug clearance. These conclusions do not support the concept that reduced intrinsic clearance contributes to age-related changes in hepatic drug metabolism.

3.3 Protein Binding

There is a slight reduction in albumin[41,42] and possibly an increase in α_1-acid glycoprotein with age.[71] The decrease of albumin has been associated with an increase in the unbound fraction of some drugs such as phenytoin,[72] diazepam[73] and piroxicam,[74] but not of prazosin,[75] warfarin[76] and verapamil.[77] It is usually conceded that any age-related effects on protein binding have little clinical significance.[11,78,79]

However, for highly bound, capacity-limited drugs, changes in protein binding can influence he-

Table VI. Influence of old age in humans on the metabolism of drugs and other compounds that undergo phase I phase II, capacity-limited and flow-limited metabolism. The numbers represent the percentage age-related changes in clearance reported in the studies presented by Durnas et al.[59]

Reduced	Change (%)	Unchanged (average)	Change (%)
Flow-limited			
Indocyanine green	–35,[a] –60[a]		
Pethidine	–44,[a] +12		
Morphine	–18,[a] –35,[a] –16		
Propranolol	–51, –41,[a] –30,[a] –24[a]		
Amitryptiline	–62,[a] –14		
Verapamil	–32,[a] –42		
Imipramine	–45[a]		
Lignocaine	+7, –35,[a] –6		
Capacity-limited			
Theophylline	–22,[a] –33,[a] –15, +33, +17, –15	Diazepam	–3
	Nonsmoker –35,[a] –33,[a] +11, –31[a]		Male –39,[a] –48[a]
			Female –6, –17
Antipyrine	–20,[a] –42,[a] +32, –39,[a] –52,[a] –51,[a] +32, –33[a]	Digitoxin	+20
		Phenytoin	+62,[a] +4
		Salicylic acid	–7, +4, –29
		Valproic acid (valporate sodium)	0, –16, 0
		Warfarin	25, –25, 0
Phase I			
Antipyrine	–20,[a] –42,[a] +32, –39,[a] –52,[a] –51,[a] +32, –33[a]	Warfarin	–25, –25, 0
Chlormethiazole	84,[a] –30[a]	Caffeine	+13
Diltiazem	–7, –39[a]	Phenytoin	+62,[a] +4
Propranolol	–51, –41,[a] –30,[a] –24[a]		
Theophylline	–22,[a] –33,[a] –15, +33, +17, –15		
	Nonsmoker –35,[a] –33,[a] +11,–31[a]		
Impramine	–45[a]		
Amitryptiline	–62,[a] –14		
Verapamil	–32,[a] –42		
Ibuprufen	–16,[a] +12		
Lignocaine	+7, –35,[a] –6		
Phase II			
Morphine	–18,[a] –35,[a] –16	Isoniazid	Rapid acetylator +13, –13
			Slow acetylator –1, –22
		Oxazepam	+20
		Paracetamol	–35,[a] –21, –25,[a] –34,[a] –23, –19,[a] –8
		Salicylic acid	–7, +4, –29
		Temazepam	Male –1
			Female –12

a Statistically significant.

Symbols: - = decrease; + = increase.

patic clearance. From equation 2, it can be seen that for any highly protein bound drug where CL_{int} is much less than Q, an increase in f_u will cause a proportionate increase in hepatic clearance. This may be of significance for the hepatic metabolism of drugs such as warfarin.

The fact that any age-related decrease in albumin will tend to increase the clearance of some drugs indicates that protein binding is not an explanation for age effects on hepatic drug metabolism. However, the albumin concentration must be taken into consideration when interpreting the effects of age on the clearance and pharmacodynamic effect of highly bound, capacity-limited drugs.

4. Disease-Based Theories of Aging and Hepatic Drug Metabolism

The age-related reduction in hepatic blood flow is associated with a reduction in the clearance of flow-limited drugs. However, the effects of age on liver size and protein binding do not appear to have a consistent effect on hepatic drug metabolism (table VI). Furthermore, the changes in Q, CL_{int} and f_u do not provide a complete explanation for the effects of age on the clearance of all drugs that undergo hepatic metabolism (table III). On the basis of such inconsistencies, Vestal[9] concluded that '. . . it is still not possible to predict in a reliable manner the clearance of drugs eliminated primarily by the liver based upon age or age-related variables such as liver blood flow or liver volume'. Therefore, it is worthwhile examining alternative, disease-based theories of drug metabolism. James[12] has commented that the elimination and disposition of some drugs in cirrhosis and advanced age are 'strikingly similar'. In their review of cirrhosis and drug metabolism, Morgan and McLean[66] considered 4 main mechanisms by which chronic liver disease could influence drug metabolism. These were:

- intact hepatocyte theory
- sick cell theory
- impaired drug uptake theory
- oxygen limitation theory.

This analysis can be usefully applied to the effects of aging on hepatic drug metabolism. The predicted effects of each of these theories on hepatic drug metabolism are shown in table V.

4.1 Intact Hepatocyte Theory

The 'intact hepatocyte' theory of liver disease states that hepatocytes function normally, but that there is a reduced mass of hepatocytes with corresponding reduction of blood flow. This theory predicts that the reduction in the clearance of flow-limited and capacity-limited drugs will be proportional and that there will be no change in the activity of enzymes measured *in vitro*.[65,80,81] The intact hepatocyte theory is functionally equivalent to the proposals that the effects of age on drug metabolism are secondary to reduced blood flow and liver size but not abnormal enzyme activity, that is, the major prevailing theory for the effects of aging on hepatic drug metabolism.[9,10,12-14,69]

The effect of age on the clearance of flow-limited drugs and capacity-limited drugs has been shown to be different in single test substrate studies. There is a uniform reduction in the clearance of flow-limited drugs but a variable effect on capacity-limited metabolism (table VI). The most useful studies, however, are those that simultaneously compare the effect of age on the clearances of a flow-limited and a capacity-limited drug in the same patient. Vestal[9] found that the age-related reduction in the clearance of the flow-limited drugs, indocyanine green clearance and propranolol did not correlate with the decrease in the clearance of the capacity-limited drug, antipyrine. This lack of correlation between the decrease in the clearance of flow-limited and capacity-limited drugs is inconsistent with the intact hepatocyte theory. Furthermore, the intact hepatocyte theory cannot explain the apparent selective reduction of clearances of drugs that undergo phase I metabolism compared with those that undergo phase II metabolism (table VI).

4.2 Sick Cell Theory

The sick cell theory states there is a global decline in hepatocyte function with disease and predicts that the free clearance of all capacity-limited drugs should decline by the same proportion (table V).[65] This type of trend with old age is not

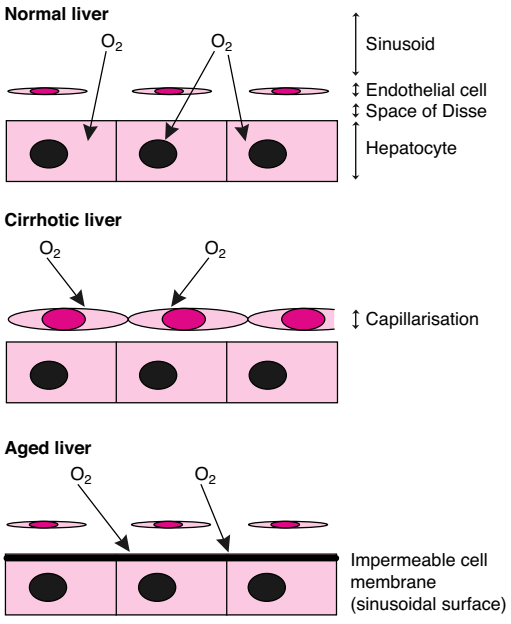

Fig. 1. Possible barriers to the uptake of oxygen in normal, cirrhotic and aged livers.

supported by the available data as shown in table VI, where it can be seen that there is considerable variability in the effects of age on capacity-limited metabolism. In addition, it has been reported[82] that the effect of age on the clearance of antipyrine and triazolam, measured in the same patients, was poorly correlated.

The sick cell theory would predict a reduction of enzyme activity measured *in vitro*. There is a reduction of phase I metabolic activity in old male rats; however, this is a species- and gender-specific phenomena *in vitro*. Phase I and II drug metabolising enzymes in humans have been reported to be unchanged and, indeed, the activities of most hepatic enzymes are not affected by aging (see sections 1.3 and 1.5).[39]

The preservation of drug metabolising enzyme activity *in vitro* and the nonproportional reduction in the clearance of different capacity-limited drugs exclude a global impairment of hepatocyte function and is at variance with the 'sick cell' hypothesis for the effects of aging on hepatic drug metabolism.

4.3 Impaired Drug Uptake Theory

The impaired uptake theory of cirrhosis states that the capillarised sinusoid provides a barrier for the passage of drugs, particularly those that are protein-bound.[83] If impaired uptake is the primary cause of impaired drug elimination, then lipid-soluble drugs with low degrees of protein binding such as antipyrine, caffeine and theophylline would be expected to exhibit a lesser reduction of elimination than highly bound substrates such as propranolol and indocyanine green.[66] Furthermore, it would be expected that aging would have a greater effect on the metabolism of less lipophilic and less diffusible drugs such as morphine and paracetamol. Any change in clearance will be easier to detect in flow-limited metabolism where the uptake is more likely to be a rate-limiting step. The results in table VI are not consistent with these required trends.

4.4 Oxygen Limitation Theory

The oxygen limitation theory was developed to explain the selective loss of phase I metabolism that accompanies cirrhosis of the liver.[65] Phase I enzymes are directly dependent on oxygen supply in contrast to phase II enzymes.[62,63] A hypoxic threshold has been identified for propranolol clearance in healthy rat livers[64] and more recently in livers with cirrhosis.[84] Usually the passage of oxygen across cell membranes is by simple diffusion and free mixing. This means that the highly fenestrated endothelium of the hepatic sinusoid does not provide any kind of significant diffusion barrier for most substances, including oxygen (fig. 1).

In cirrhosis, the endothelium becomes more like that seen in other capillary networks ('capillarised') and could provide a barrier to the diffusion and, hence, uptake of oxygen (fig. 1).[65,66] The oxygen limitation theory is supported by the work of Goresky and associates who found that there is no barrier to oxygen uptake in the normal liver,[85]

while hepatic theophylline clearance in rats with cirrhosis is restored to the normal range by oxygen supplementation.[86] In the heart, where there is a capillary network, oxygen permeability is similarly reduced.[87]

Is there any evidence for an age-related oxygen limitation? First, there are similarities between drug metabolism changes in cirrhotic and aged livers,[12] and in particular, both appear to have a selective impairment of the clearance of drugs that undergo primarily phase I metabolism. In fact, a requirement of the oxygen limitation theory is that phase I metabolism is a gauge of oxygen availability in the hepatocyte. However, it should be noted that the influence of age on phase I metabolism has been controversial.[10]

Even though *in vitro* activity of phase I enzymes does not change with age, nearly all drugs metabolised by phase I pathways have reduced clearance in old age (table VI). These include many flow-limited drugs where clearance is reduced secondary to blood flow changes, as well as capacity-limited drugs such as theophylline and antipyrine. Two of the exceptions, phenytoin and warfarin, are potentially altered by protein binding effects which may cause a compensatory increase of clearance. The other exception is caffeine. In their review, which was used as the basis for this analysis, Durnas et al.[59] included only 1 study and this reported no change in caffeine clearance with aging.[88] Another group has reported a significant 35% decrease in caffeine clearance in older individuals.[21] In their recent review, Kinirons and Crome[10] analysed reports of the effects of age on CYP activity that had been measured *in vivo*. Of the 8 isoforms where results were available, 6 were reduced with age and only 2 (CYP2D6 and CYP2A) were unchanged.

An age-related hepatocyte diffusion barrier for oxygen would explain the apparent paradox that experiments using liver microsomes, where oxygen delivery is not constrained, show no reduction of phase I enzyme activity whereas *in vivo* drug clearance studies appear to show a decline in phase I metabolism. A parallel aging hypothesis based on oxygen restriction has been reported for the human testes. Age-related changes in testicular steroid metabolism *in vivo* were reproduced with reduced oxygen supply during *in vitro* incubation experiments with testicular tissue.[89]

Oxygen uptake has been reported to be reduced in the livers of aged rats. Handler et al.[38] reported that oxygen uptake in perfused livers was reduced from 121 ± 5 μmol/g/h in rats aged 3 to 6 months to 75 ± 8 μmol/g/h in 22 to 24 month rats. We also have observed a reduction of oxygen uptake in perfused livers from 1.15 ± 0.19 μmol/g/min in young rats ($n = 23$) to 1.03 ± 0.22 μmol/g/min in rats aged 24 to 28 months ($n = 18$, $p = 0.03$, unpublished data).[90] On the other hand, no age-related change in endogenous respiration was observed across the hepatomesenteric vascular bed in humans.[91]

Even though the sinusoids of the aged liver are not capillarised,[92] other diffusional barriers could develop with age, ranging from altered extracellular matrix to alterations in the hepatocyte itself, including, in particular, the hepatocyte surface membrane. Because of the age-related decrease in steady-state transfer capacity for carbon monoxide.[93] the transport of oxygen in the lung is assumed to be diffusion limited in elderly people. While the site of the diffusional barrier (alveolar lining, basement membrane or capillary endothelium) has not been determined, reductionist logic would implicate the extracellular matrix or the alveolar cell membranes as analogous candidate sites applicable to the liver.

There have only been a few reports of the effects of age on hepatocyte membrane permeability. Therefore, we reanalysed the results of a study published previously. In this study,[48] the multiple indicator-dilution technique was used to investigate intracellular pH (pH_i) in the perfused livers of aged rats (24 to 28 months). The indicator used to measure pH_i was dimethadione. This small molecule passes across the hepatocyte membrane by diffusion[94] and is a possible surrogate marker for oxygen permeability. The data were reanalysed using the physiological techniques derived by Goresky to determine the permeability-surface area product

for dimethadione (PS_{DMO}), as described previously.[94] This was significantly reduced in the livers of aged rats [8.6 ± 1.3 ml/g/sec (n = 5, 2 to 3 months old) *vs* 3.74 ± 2.04 ml/g/sec (n = 4, 24 to 28 months), p = 0.003] (fig. 2).[90,95] Thus, aging is associated with membrane changes that impair diffusion of small molecules. PS_{DMO} in young rats fell to the same concentration seen in the aged rats after reoxygenation injury (fig. 2). This raises the possibility that the age-related effects on hepatocyte membrane permeability could be secondary to injury by free radicals.

Age-related changes in cell geometry provide another possible mechanism for impaired delivery of oxygen to intracellular enzymes. Aging in some electron microscopy studies is associated with swelling of hepatocytes (section 1.1). Such increases in cell size will increase path length and reduce oxygen delivery according to Fick's first law of diffusion.

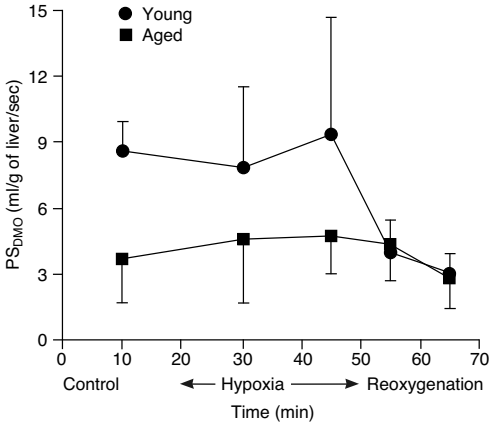

Fig. 2. The effect of age on permeability-surface area product of dimethadione (PS_{DMO}) in the perfused rat liver. The initial measurements were performed under normal conditions. Then, the livers were rendered hypoxic for 30 minutes and reoxygenated for 15 minutes. PS_{DMO} was significantly higher in the young rats under normal perfusion conditions but fell to the same value seen in old rats after reoxygenation.

5. Alternative Theories

5.1 Hepatocyte Membrane Transporter Change

Aging is associated with hepatocyte membrane changes which could affect drug uptake. In fact, Zs-Nagy[96] has hypothesised that such changes may be central to the aging process. The hepatocyte membrane contains more cholesterol and become less fluid with age.[69] This is associated with, and may be the cause of, impaired activity of Na-K ATPase[97] and membrane uptake of rubidium,[98] ouabain,[99] glucose,[27] nicotinic acid,[100] taurocholate and thymidine.[101] There does appear to be a barrier to the uptake of some substances by the aged liver which indicates that this theory warrants further investigation.

5.2 Cellular Physico-Chemical Change

It has been recognised that cellular physicochemical parameters may be an important influence on enzyme activity. For example, Zs-Nagy and associates[96,98] have reported that aging is associated with altered intracellular osmolality. Reduced membrane fluidity may influence the activity of CYP enzymes which are associated with the membranes of the endoplasmic reticulum.[69] Intracellular pH tends to be more alkaline in the livers of aged rats but is unchanged after fasting.[48] All of these parameters do change with age, and hence can potentially influence enzyme function, but their effects will not be observed in experiments performed *in vitro* with purified enzyme preparations.

6. Dosage Guidelines

Certain principles emerge from this review which provide mechanistic explanations for dosage adjustments required in elderly people to account for changes in liver clearance. The review of simple drug clearance studies performed to date indicates that in most cases it is prudent to reduce the dose of drugs that undergo phase I metabolism and/or flow-limited metabolism in elderly patients. Reduced clearance of flow-limited drugs is proportional to the reduction in hepatic blood flow which provides a logical mechanism. The extent of this change is in the order of 40%, accordingly the

doses of high clearance drugs should be routinely reduced by this amount. The dose of drugs metabolised by redox reactions should be reduced by approximately 30% while that of drugs eliminated following conjugation need not be changed. A general preference for drugs subject to conjugation might reasonably be advocated. Definitive dosage recommendations depend on full exposition of age-related changes in pharmacodynamics and extrahepatic drug disposition.

7. Therapeutic Horizons and Conclusions

The paradox of impaired phase I clearance *in vivo* and preserved enzyme activity *in vitro* is difficult to explain using current theories. However, by analogy with our understanding of drug metabolism in liver disease, it can be speculated that a hepatocyte diffusion barrier to oxygen develops with age. This provides a plausible explanation for the paradox and has some experimental support. Again, by analogy with the cirrhotic liver, it may be possible to normalise the function of the aged liver by improving hepatic oxygenation.[65,66]

The areas of uncertainty in our suggested conclusions reflect the paucity of elements of the database in the area. There is an obvious need to perform studies of the type reported by Vestal and associates[9,60] extended to dual administration of low clearance marker drugs subjected to phase I and II metabolism. There is a need to explore substrate exchange process within the liver and within the hepatocyte. Therapeutic considerations indicate the need to test the hepatocyte diffusion barrier hypothesis presented here, and in particular, to test the application of the principles enunciated for improving hepatic oxygenation as applied to cirrhotic liver disease.[65,66]

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