The occurrence of feminization and hypogonadism in men with chronic liver disease, and recent reports of hepatic tumours arising in women using oral contraceptive preparations, have served to interest clinicians in the relationship between the liver and sex hormones. These two clinical observations pose different but interrelated questions. First, what is the role of the liver in sex steroid metabolism and how does hepatic disease alter this, and, second, what is the normal action of sex steroids on liver function and how is it modified by exogenous, or elevated levels of endogenous, sex hormones? The brief summary of the physiology of sex hormones pertinent to those biochemical changes observed in chronic liver disease which follows is limited to male physiology. Despite the increase in alcoholic liver disease seen in females over the last decade changes in their sex hormone metabolism have received almost no attention.

The testicular Leydig cells of the normal adult male secrete about 4-9 mg of testosterone/day and this is the major circulating androgen [1]. The weaker androgen, androstenedione, is secreted by both the testis and adrenal gland (about 1-2 mg/day) but, like other adrenal androgens such as dehydroepiandrosterone, its activity is probably mainly attributable to peripheral conversion into testosterone [2].

Recent studies on the plasma protein binding of sex hormones may have important implications for liver disease. Most testosterone (and oestradiol) circulates bound to plasma proteins, either albumin (low affinity, unsaturable binding) or sex hormone binding globulin (SHBG, high affinity, saturable binding). Only a small fraction exists free of all plasma proteins. This 'free' steroid, estimated by equilibrium dialysis to be, in the case of testosterone, about 2% of the total has been widely considered to be the biologically active fraction [3]. However, this view is now being questioned. It appears that in some situations it may be the fraction of testosterone not bound to SHBG (i.e. ‘free’ + albumin bound) and total oestradiol which are important [4]. Iqbal et al. [5] have recently developed a computerized technique to derive the relative amounts of sex hormone bound to albumin, SHBG, and free from either, from the total hormone and SHBG concentrations, which should make investigation of this problem simpler. The plasma concentration of SHBG, which is presumed to be synthesized in the liver, and has a single common binding site for oestradiol and testosterone [6], correlates positively with oestradiol levels and negatively with testosterone, suggesting that these are major factors controlling synthesis. The correlation in the case of testosterone is best with the free fraction whereas with oestrogen the best correlation is with the total amount, supporting the contention that the total rather than free fraction of oestrogen is, at least in this situation, biologically important [7]. The metabolic clearance rate of testosterone and oestradiol are inversely related to the SHBG concentration [8]. Serum albumin is not of importance in determining the metabolic clearance rate of these steroids [5].

Testosterone may be metabolized by peripheral conversion into its more active metabolite dihydrotestosterone (DHT, 5α reduction), or into oestrogens, or by degradation in the liver. The conversion into DHT occurs at the site of action and is a prerequisite for the activity of testosterone in several tissues, particularly the skin and male reproductive tract. In the liver, which is responsible for clearing about 50% of testosterone entering the splanchnic circulation during the first pass in males (and up to 100% in normal females) [9], the lipophilic steroids including testosterone are rendered water soluble by reduction of the double bonds in the steroid nucleus. The introduction of hydroxyl groups act as loci for the further introduction of hydrophilic groups. The main metabolites, androsterone and etiocholanolone (the products of 5α and 5β reduction), and a minor amount of testosterone are secreted as
androgens are converted into oestrogens (by the small fraction, perhaps 1%, of circulating glucuronides and sulphates in the urine. Only a small amount is secreted by the testis [12, 13]. These oestrogens are metabolized mainly within the liver by 2- or 16α-hydroxylation to metabolites such as 2-methoxyoestrone and oestrone which are secreted, after conjugation, into bile. Biliary oestrogens undergo an enterohepatic circulation [10].

Clinical features and biochemical basis of hypogonadism

Most men with chronic liver disease have clinical and biochemical evidence of hypogonadism. Lack of libido and impotence are the major complaints and are associated with decreased testicular size, histological evidence of testicular atrophy and loss of body hair [16-19]. Total testosterone is within the lower part of the normal range in men with well compensated liver disease but falls with disease progression [17, 20-23]. There is a several-fold increase in SHBG [4, 17, 24, 25], which is surprising in view of its presumed hepatic synthesis. This leads to a decrease in the metabolic clearance rate of testosterone [2, 17] and a marked reduction in the free fraction [17, 21, 26, 27]. The metabolic production rate, however, falls to about a quarter of the normal value [1, 2, 17] and up to 15% of testosterone is derived from androstenedione rather than the 1% in normal males [2]. Free testosterone in men with cirrhosis is thus low despite an increased contribution from androstenedione and a decrease in the metabolic clearance rate. The finding of normal levels of luteinizing hormone (LH) in this situation has led Van Thiel et al. to propose that, in addition to primary testicular failure, there must also be a hypothalamic-pituitary defect, otherwise LH levels should rise [19]. However, in later studies [16, 28, 29] these and other workers did find LH levels elevated; the nature of any hypothalamic-pituitary defect thus remains unclear.

The mechanism of these changes is surrounded by considerable controversy. In the U.S.A. there has been a tendency to attribute hypogonadism directly to excessive alcohol consumption (which is the commonest cause of cirrhosis in the Western world), whereas in Europe the resultant liver disease has been implicated. Certainly alcohol (or its metabolites) is a direct testicular toxin in vitro [30], causes testicular atrophy in an animal model [31], and Boiesen et al. found that testicular damage (particularly to the germinal epithelium) in alcoholic men was independent of the presence or absence of chronic liver disease [32]. Ylikahri et al. reported that within 20 h of ingestion of 1.5 g of alcohol/kg over 3 h testosterone levels had fallen by 50% [33], and Gordon et al. that the fall in testosterone persisted for 1 month while volunteer men were given 3 g of alcohol/kg per day [34]. On the other hand outside the U.S.A., where it is still relatively common to find men with cirrhosis in whom it is possible to exclude alcohol as an aetiological factor, there is no doubt that such patients may exhibit all the clinical signs, symptoms and biochemical derangements of hypogonadism and feminization [17, 35, 36]. Green has emphasized that, apart from a fall in testosterone, the acute effects of alcohol described above are quite different from those seen in chronic liver disease [37].

In an attempt to separate the role of alcohol and chronic liver disease Valimaki et al. [29] have compared the hormonal status of 13 male alcoholics with, and 16 without, cirrhosis. Low testosterone and raised LH and oestrone occurred significantly more frequently in those with cirrhosis, emphasizing the role of underlying liver disease [29]. On the other hand, Van Thiels et al. [28] compared the abnormalities in sex hormone metabolism in a group of alcoholic men and a group of haemophiliac patients with apparently similar degrees of liver dysfunction. The alcoholic patients had significantly lower testosterone, spermatozoa concentrations and seminal plasma volume, whereas those with haemophilia had normal values. In several other aspects of pituitary-gonadal function the two groups also showed marked differences, supporting the author's argument that factors other than liver disease are important in producing hormonal abnormalities [28].

Clinical features and biochemical basis of feminization

Gynaecomastia is the most striking manifestation of hormonal abnormalities in men with chronic liver disease, and 'arterial spiders' and liver palms are also considered evidence of feminization. It occurs in between 15 and 40% of cases, most often in patients with alcoholic cirrhosis [16, 17, 26, 35, 36]. Edmondson and coworkers [38-40]
suggested that chronic liver disease might lead to high levels of oestrogens consequent upon failure of the liver to metabolize endogenously produced steroids, and supported this idea by showing low urinary androgen and high urinary oestrogen excretion. With the advent of radioimmunoassays several workers took the opportunity to test Edmondson's hypothesis directly and reported that serum oestradiol ranged from normal [26, 41] to markedly raised [21, 42], though elevation of oestrone was a consistent finding [41-43]. Increased peripheral conversion of testosterone into oestradiol [16] and adrenal androstenedione into oestrone [42, 44, 45] have been documented and attributed to portosystemic shunting in the cirrhotic patients [46]. There is also evidence in rats that alcohol may increase hepatic aromatase activity [47] and adrenal androstenedione production [48]. In agreement with these findings the plasma production rate for oestrone and oestradiol have been shown to be elevated [2, 49].

Most reviewers have concluded that the observed increases in oestradiol are not large enough to account for the feminization seen in cirrhosis, particularly since the results of free oestradiol estimations have been equally varied and oestrone is only a weak oestrogen. However, the reservations about the biological relevance of free oestradiol should be recalled and when Kley et al. [20] classified 15 alcoholic patients into three groups ranging from group 1 (well compensated, no ascites, no oesophageal haemorrhage and no signs of feminization) to grade 3 (decompensated, ascites, variceal haemorrhage and gynaecomastia with testicular atrophy), the progressive rise in oestradiol was marked, as was the fall in free testosterone (Table 1). Furthermore the pronounced rise in SHBG which occurs in chronic liver disease tends to tilt the oestrogen/testosterone ratio in favour of the former because testosterone has a significantly higher affinity for SHBG.

Table 1. Concentrations of free testosterone and free oestradiol in plasma in healthy males (n = 8) and in patients of the same age with alcoholic induced cirrhosis of the liver

<table>
<thead>
<tr>
<th>Group</th>
<th>Free testosterone (pg/ml)</th>
<th>Free oestradiol (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>124.7 ± 10.2</td>
<td>0.51 ± 0.06</td>
</tr>
<tr>
<td>Group 1</td>
<td>94.9 ± 9.9</td>
<td>0.55 ± 0.03</td>
</tr>
<tr>
<td>Group 2</td>
<td>57.8 ± 7.4</td>
<td>0.71 ± 0.05</td>
</tr>
<tr>
<td>Group 3</td>
<td>32.0 ± 4.0</td>
<td>1.14 ± 0.15</td>
</tr>
</tbody>
</table>

Perhaps the commonest cause of gynaecomastia in cirrhosis is the treatment of ascites with spironolactone. The biochemical abnormalities induced by this drug are strikingly similar to those seen in cirrhosis. Testosterone is decreased, oestradiol is increased and the rate of conversion between the two is also increased. Spironolactone may also block the androgen receptor in the male breast, leading to the unopposed action of oestrogen [52].

The complexity of the factors involved and the lack of agreement between different workers on major points such as whether or not oestrogen levels are raised has led some to despair of an unifying hypothesis. If increased oestrogens are responsible then they are not, as initially suggested by Edmondson, caused by decreased hepatic inactivation but rather by increased production. Possible explanations include alcohol stimulated local (i.e. breast), or general aromatization of androgens which have escaped hepatic metabolism because of portosystemic shunting [46]. Other metabolites of oestradiol such as 16α-hydroxy-oestrone are increased in cirrhosis and there is evidence that they may have marked oestrogenic capacity [53]. The possibility that breast tissue in the male cirrhotic is more sensitive to oestrogen should also be considered. The biological activity of oestrogen is a function not only of its plasma level but also the receptor concentration in the target tissue (and their strength of binding). Eagon et al. have shown an increase in oestrone receptor concentration in the liver of rats fed with alcohol and if the phenomenon could be extended to other organs such as the male breast it would provide an attractive explanation of the feminization of males with alcoholic liver disease [54].

Sounder reasons for doubting Edmondson's hypothesis are that the metabolic clearance rates of oestradiol and oestrone are usually quite normal in male cirrhotic patients [2, 17, 42] and the correlation between elevated oestrogen levels and gynaecomastia is poor. Several other clinical parallels occur, however, such as Klinefelter's syndrome, where gynaecomastia is also frequent despite normal or only marginally elevated oestrogen levels [50]. Furthermore, the histological features of gynaecomastia in cirrhotic patients are reported to be different from those in men taking oestrogens. Schwartz & Wilens [51] showed that formation of mammary gland structures including acinarlobules was seen in eight of 28 cases associated with prolonged oestrogen therapy but in none of 30 cases due to cirrhosis.

Schwartz & Wilens [51] showed that formation of mammary gland structures including acinarlobules was seen in eight of 28 cases associated with prolonged oestrogen therapy but in none of 30 cases due to cirrhosis.
same workers have also shown that low testosterone levels induced by castration increased oestrogen receptor activity [55].

As with hypogonadism, where the biochemical basis is better established, the confusion between the relative roles of alcohol and chronic liver disease persists. To-date it must be said that the school implicating alcohol as the prime factor have produced the most supportive evidence. The biochemical techniques to answer this question are now widely available, it is well devised clinical studies which are required. At present it seems that both alcohol excess and chronic liver disease may cause hypogonadism and feminization and that when the two occur together the effects are compounded.

**Physiological effects of sex hormones on the liver**

In the classical experiments of Jensen & Jacobson [56] labelled oestradiol was injected into female rats and the changes in organ distribution were measured over several hours. In uterus and vagina the uptake increased rapidly during the first 2 h and 50% of the maximum activity was still present at 6 h. By contrast, maximum activity in the liver was achieved at 15 min, after which it fell rapidly to reach 50% before 1 h. The prolonged and active retention by uterus and vagina led to the concept of oestrogen receptor proteins (see below) and was consistent with these two organs being oestrogen responsive whereas tissues such as liver were not. Despite this, several authors now consider the liver to be a sex hormone target tissue. The evidence for this contention came initially from the observation that the activity of steroid and drug metabolizing enzymes and the serum concentrations of several hepatic proteins was different in males and females [57]. It was further strengthened by reports that rises in endogenous oestrogens during pregnancy or administration of exogenous oestrogens (oral contraceptive steroids) also led to similar changes. Thus serum levels of α1-antitrypsin, ceruloplasmin, renin substrate, transferrin and the hormone binding proteins (sex hormone, cortisol and thyroid binding globulins) all increase strikingly during pregnancy [58–63].

It is only recently, however, that the mechanism of these interactions has been investigated. The most widely accepted model for sex steroid action is that the steroid hormone first binds to a specific soluble cytoplasmic protein, the 'steroid receptor', after passive diffusion across the cell membrane. The hormone–receptor complex then translocates to the nucleus where it interacts with a nuclear acceptor site, leading to messenger RNA production and the appropriate phenotypic effect [55]. Human liver as well as that of several other mammals contains cytoplasmic and nuclear oestrogen binding proteins with similar properties to those found in breast and uterus [64–69]. A second class of steroid binding protein characterized by a higher capacity for binding both oestrogens and androgens has recently been described in rat liver [54, 55, 69, 70]. However, the demonstration of such receptors should not necessarily be taken as providing a mechanism for the effects of oestrogen on the liver. Pietras & Szego [71] have reported that although there was indeed rapid nuclear binding in intact hepatocytes exposed to oestradiol, the initial binding was at least partially to the hepatocyte plasma membrane, and suggest that the apparent cytosolic binding may be an artifact of the homogenization procedures and that several cellular loci, including microsomal, lysosomal and plasmalemmal as well as cytosolic elements may be involved in the nuclear translocation circuits. DHT, oestradiol and progesterone may exert their actions directly on liver microsomes, where binding sites specific for these steroids have recently been demonstrated in rat liver [72, 73]. Studies such as these and others described below have led Eriksson to conclude "so far no data have been published which clearly demonstrate a receptor dependent oestrogen response in the rat liver" [68].

Although testosterone increases liver weight and protein content as part of its general 'anabolic' action, such changes are not specific to testosterone and require large doses, leading several workers to question whether androgens act on the liver by classical receptor mediated pathways. Some have not been able to detect cytosolic androgen receptors in rat [74] or human liver [75] and others have provided evidence that the well documented effect of androgens on the hepatic synthesis of rat α2u-globulin is receptor mediated [76]. Brown et al. [76] investigated the stimulatory effect of testosterone on hepatic weight, microsomal protein concentration and ethylmorphine-N-demethylase activity. Only the latter was blocked by the anti-androgen flutamide, leading the authors to conclude that testosterone, according to the actions studied, may act via receptor dependent or independent pathways [76].

Caution should also be exercised before attributing sex differences in hepatic function to a direct effect of these steroids on the liver. Many sex steroid metabolizing enzymes such as the hydroxylases are more active in the male rat liver, though 5α-reductase is more active in the female [56, 77]. Early studies showed that these sex differences
could, in some cases (e.g. 6β-hydroxylase active on androstenedione) be abolished by castration of the adult animal and in other cases (e.g. 2α-hydroxylase active on pregnenedione) only by neonatal castration, suggesting that the enzyme system was 'imprinted' by neonatal exposure to androgens [77, 78]. In both cases androgen administration could to some extent restore the sex differences. Subsequently it became evident that these effects could be demonstrated only in the presence of an intact hypothalamic-pituitary system, leading to the hypothesis that androgens act not directly on the liver but by inhibiting the pituitary secretion of a feminizing hormone in the adult male [79, 80]. Growth hormone or prolactin seem the most likely candidate hormones. Although absolute levels of growth hormone are similar in males and females, their pattern of secretion appears to differ. Most recently, Norstedt et al. have shown that lesions in the anterior hypothalamic-pituitary area caused feminization of hepatic steroid metabolism in male rats and provided evidence that somatotrophin might be the central neuro-endocrine mediator of these sex differences [81]. Interestingly, it has also been found that sex differences in the concentration of hepatic prolactin receptors, which are low or absent in male rats, and oestrogen receptors in regenerating male rat liver are also dependent on an intact hypothalamic-pituitary system [68] as is the previously mentioned effect of androgens on the synthesis of α2u-globulin [82, 83]. The recognition of a hypothalamic-pituitary-hepatic axis is an exciting development, the clinical relevance of which awaits investigation.

Pathological effects of sex hormones on the liver

Natural and synthetic oestrogens cause cholestasis in normal individuals [84]. Synthetic analogues of testosterone (such as methyltestosterone) also cause cholestasis but testosterone itself and progesterone do not. From a clinical point of view this assumes importance in pregnancy [85], oral contraceptive usage [86] and during treatment with drugs such as methyltestosterone [87], after which a spectrum of abnormalities ranging from subclinical cholestasis (as revealed by abnormal bromosulphthalein retention) through pruritus to frank jaundice may occur. Oestrogens may worsen the jaundice in Dubin–Johnson syndrome (a congenital hyperbilirubinaemia) [88] and there are reports of primary biliary cirrhosis presenting during pregnancy. A C-17 methyl substitution and a phenolic A ring are the most characteristic features of steroids causing cholestasis [89, 90], but host factors are also important. Jaundice of pregnancy is much more frequent in some parts of the world, such as Chile, and women who develop this condition invariably become jaundiced after using the contraceptive pill [91].

The association between benign hepatic tumours and the contraceptive pill was first described in 1973 by Baum et al. [92] and, after some initial scepticism, several hundred further reports of such tumours, which were previously considered rare, followed. About half the tumours are classified as focal nodular hyperplasia, and half hepatocellular adenoma. The risk of tumour development or, more accurately, of tumour presentation increases with increasing duration of usage (the average time of steroid exposure before presentation is about 6 years) and tumour regression with drug withdrawal has been described [93, 94]. Similar tumours have also been reported in men taking anabolic androgenic steroids and again a minimum period of 3 years seems to be required. In both instances, although microscopically some tumours may have features of malignancy, they seldom metastasize or infiltrate and this benign clinical behaviour is reflected in survival, the mortality of about 5% being attributable to intra- or extra-hepatic haemorrhage. These side effects of the contraceptive pill should not perhaps have been so surprising. Leonard [95] records that "In 1966 Dr Bosner reported to the Committee (on Safety of Drugs) that dosage with mestranol was associated with the production of nodules in the liver of rats which histologically showed various stages of nodular hyperplasia and the production of hepatoma". Subsequent studies for the Committee showed that, for example, ethynylestradiol caused benign tumours in 15.3% of male rats and 23.5% of female rats, compared with figures of 0% and 8% in control animals.

Malignant liver tumours are much less strongly associated with sex steroid compounds and despite several case reports [96] many workers now consider the association fortuitous. Nonetheless males develop hepatocellular cancer in the cirrhotic liver much more frequently than females [97]. Furthermore, in animal models male castration decreased the incidence of spontaneous hepatocellular carcinoma from 33 to 12%, and administration of testosterone to mice with chemically induced hepatic nodules significantly increased the rate of malignant transformation [98]. A particularly interesting observation was that of Goodall & Butler, who showed that whereas control rats fed with a diet of aflatoxin B1 (4 p.p.m.) consistently developed hepatic tumours, hypophysectomized animals were resistant [99]. Recently Moss and colleagues have...
found that the pathway of aflatoxin metabolism leading to the presumed carcinogenic intermediate was much more active in male than female rats [100].

The mechanism of tumour development remains unknown. The previously mentioned sex differences in steroid and drug metabolism in males and females is an obvious possibility. There are reports that primary hepatic tumours may contain androgen receptors but, as normal liver tissue also does [101], this may not be surprising. More recently, evidence has been presented that malignant liver tumours contain androgen receptors, whereas normal liver does not [75]. Limitation of space has precluded discussion of several other interactions between the liver and sex hormones, such as the increased incidence of gallstones in women using oral contraception and the effects of oestrogens on Kuffer cell function. However, it must be apparent that the gulf between our understanding of physiological and pathological effects of sex hormones on the liver is still enormous. That the liver is clearly a sexually dimorphic organ cannot be without morphologic relevance.

References


association between benign hepatomas and oral contraceptives. 


