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Testosterone and alcoholic cirrhosis

Epidemiologic, pathophysiologic and therapeutic studies in men

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INTRODUCTION

Alcoholic cirrhotic men often present testicular atrophy, azoospermia, reduced body hair, beard growth, and prostatic size, as well as gynecomastia, arterial spiders, female escutcheon, female body habitus and sexual dysfunction. These symptoms suggest an endocrine imbalance of sexual hormones, but both normal and decreased plasma testosterone (T) concentrations and normal and increased plasma oestrogen concentrations have been observed (1, 2, 3). Further, alcoholic cirrhotic men have decreased albumin and increased sex hormone binding globulin (SHBG) concentrations (1, 2, 3), which may alter the metabolism and action of sex steroids (4). The mechanisms producing the decreased T concentrations are incompletely understood, ethanol toxicity and/or liver damage being possibly involved (5, 6).

Hall and Korenchewsky (7) suggested a therapeutic value of T in liver patients. They observed an increased liver weight after injection of T propionate into castrated male rats (8). However, contradictory clinical data have been presented regarding a beneficial effect of anabolic-androgenic steroid treatment of cirrhotic patients (9).

The aims of the present study were to examine

a) plasma (or serum) T concentrations in men with alcoholic cirrhosis,
b) the pathogenic mechanisms leading to variation of plasma T concentrations,
c) the effect of oral T treatment on sex steroid concentrations in peripheral plasma, and
d) the effect of oral T treatment on survival, liver biochemistry, morphology, haemodynamics, and function, and general well-being including sexual function of alcoholic cirrhotic men.

Testosterone metabolism in normal men

The metabolism of T depends on, among other things, the metabolism of other androgens, gonadotrophins and oestrogens.

Production and interconversion of androgens. The secretion of T from the testis is controlled by luteinizing hormone (LH) and possibly indirectly by follicle stimulating hormone (FSH) (10, 11, 12). Both are secreted in bursts from the pituitary gland (10, 13). The Leydig cells secrete about 95% of plasma T (about 7 mg T/24 h), 50% of plasma 5α-dihydrotestosterone (DHT) and 20-30% of plasma androstenedione (14, 15), the remainder originating from interconversion of T or from adrenal secretion (14) (Fig. 1). Interconversion of androgens is possible in the splanchnic circulation, but the splanchnic circulation metabolises and clears androgens from plasma (14).

Protein binding of testosterone. Plasma T circulates freely (2%, non-protein bound T) or bound to albumin (55%) or SHBG (40%), or to other proteins (5%) (4, 14, 16, 17, 18). The non-protein bound T and the non-SHBG bound T (consisting mainly of non-protein bound and albumin bound T) are considered the biologically active (4, 16, 19, 20, 21), as albumin binds T with low affinity opposed to SHBG (4, 14, 16, 17, 18).

Catabolism of androgens. The systemic metabolic clearance rate of DHT is about 470 ml plasma/min compared to 700 ml plasma/min for T and 1600 ml plasma/min for androstenedione, which reflect the association constants of SHBG for the androgens (14). Women and hyperthyroid patients, who have higher SHBG concentrations, have lower systemic metabolic clearance rates of T than normal men (14). By infusion of T, the systemic clearance rate of T increased (22). The splanchnic extraction of T has been estimated to be about 50% (23). Because this extraction is greater than the non-protein bound T fraction, a major part of T removed in the splanchnic circulation is derived from protein bound T. Although the gastro-intestinal mucosa may metabolize T (24), it is generally assumed that the liver is the major determinant of the splanchnic clearance of T. In accordance, the extraction of T in the gastrointestinal tract is about 10% according to preliminary observations *(C. Gluud, F. Burcharth, P. Bennett,* unpublished observations). Experimental studies have demonstrated that non-protein bound as well as non-SHBG bound T gain freely access into liver cells, while SHBG inhibits the transport of T into the rat liver (16). *Pardridge* (16) demonstrated a 90% first-pass extraction of T in the isolated perfused rat liver both when T was injected in Ringer's solution or dissolved in rat serum (containing albumin, but no SHBG). In accordance, we *(C. Gluud, P. Bennett,* and *K. Winkler,* unpublished observations) observed a splanchnic extraction of T of about 90% after injection of T intravenously into a boar. Like rats, the boar does not have measureable amounts of SHBG (25, *C. Gluud* and *P. Bennett,* unpublished observations).

Production and interconversion of oestrogens. Oestrogens originate from testicular secretion and from enzymatic (aromatase) conversion of androgens (Fig. 1). Aromatization of T and androstenedione accounts for more than 70% of oestradiol and more than 90% of oestrone produced (26, 27), and >90% of aromatization takes place in extrasplanchnic tissues (27). Oestrone and oestradiol are readily interconverted (26).

Plasma protein binding of oestrogens. The non-protein bound fraction of oestrogens accounts for 2-3%, the albumin-bound fraction for 75%, and the SHBG-bound fraction for 25% (17, 18). Due to a lower association constant of SHBG for oestradiol, the latter gains faster access to the extravascular pool than T (4, 16, 26, 28, 29).

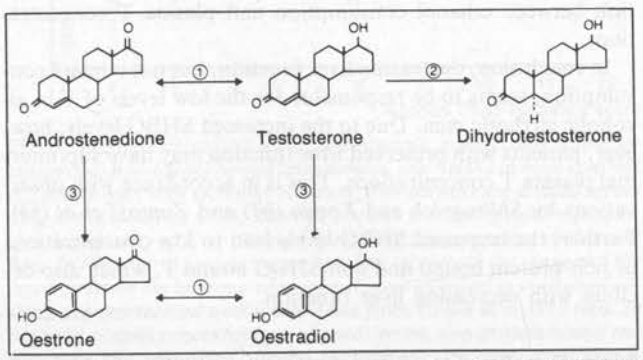


Fig. 1. *Interconversion of androgens and conversion to oestrogens. Enzymes involved are: (1) 17-ketosteroid oxidoreductase, (2) 5α-reductase, (3) aromatase.*

Determination of plasma testosterone concentrations

Plasma T (or serum, giving identical results (30)) can be measured with sensitive and specific radioimmunoassays (RIA) (31, 32). However, antibodies raised against T may react with other steroids and other substances (e.g. lipids) may interfere (31, 32, 33). Therefore, separation techniques must be used (31, 32). We have used a modification of the RIA described by *Barberia* and *Thorneycroft* (34), the major modification being the use of a different elution system of the Celite column chromatography (35, 36, 37, 38, 39, 40, 41). The detection limit is 0.20 nmol/l and the RIA is specific. Thus, the addition of interfering steroids to the samples do not affect the analytical results, and the recovery of T is 96% (SD = 4%) *(P. Bennett,* unpublished observations). Further, when the RIA was compared to another RIA using other methods for extraction, seperation, and antibody production, we found no significant differences between the T values of the assays in men with alcoholic liver disease and alcoholic cirrhosis (39) (Fig. 2). By measurement of SHBG and albumin, it was made probable that the non-protein bound T concentration could be calculated in alcoholic patients together with the non-SHBG bound T concentration (39).

In a further study (42), we used the RIA of *Parker et al* (43) for determination of T. This RIA obtains plasma T concentrations in the same range as those obtained by the former method (39) both when normal men and men with alcoholic cirrhosis were examined (42) (Fig. 3).

Plasma testosterone concentrations in men exposed to ethanol

Van Thiel et al (44) suggested that the low plasma T levels of men with alcoholic liver disease were due to a direct effect of ethanol and not secondary to an effect of ethanol on the liver. Since then *in vitro* and *in vivo* studies have shown that ethanol exerts multiple effects on the hypothalamic-pituitary-gonadal system. The general concept that has emerged is that ethanol (or acetaldehyde) depresses T levels (2, 3, 45, 46, 47, 48, 49). However, *in vitro* experiments should be interpreted with caution as they do not compare to the intact organism in which metabolic and hormonal factors may counteract a toxic effect of ethanol. Second, animal experiments may not be relevant for

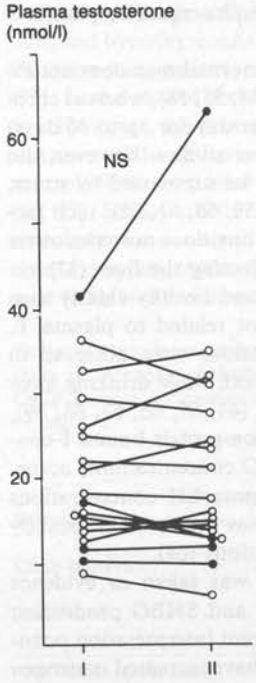


Fig. 2. *Plasma concentrations of testosterone in alcoholic cirrhotic men treated with testosterone until the day before blood was drawn (•, n = 4) or previously untreated (○, n = 14). Testosterone concentrations were determined by two different methods, method II being that used in the present study (Data from* Gluud *and* Bennett *1986 (39)). NS denotes not significant (Wilcoxon test). The results correlated significantly (R = 0.91, p < 0.001, Spearman test).*

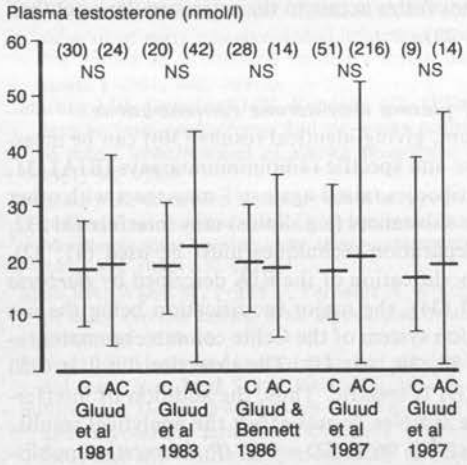


Fig. 3. *Plasma testosterone concentrations in male controls (C) and men with alcoholic cirrhosis (AC) from five series (references 35, 37, 39, 40, 42). Vertical lines represent range and horizontal lines median. Figures in brackets are number of subjects. NS denotes not significant (Mann-Whitney test).*

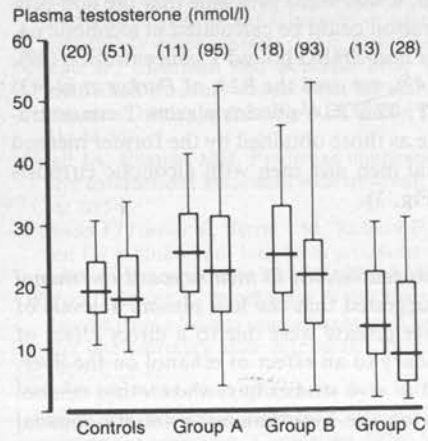


Fig. 4. *Plasma testosterone concentrations in male controls and men with alcoholic cirrhosis divided into groups A, B and C according to the modified Child-Turcotte's classification. Data from* Gluud et al *1983 (37) (left) and* Gluud et al *1987 (40) (right). Figures in brackets are number of subjects. Vertical lines represent range, boxes interquartile range, and horizontal lines median.*

man due to species differences (50, 51). Third, data in men exposed to ethanol display conflicting results regarding plasma T concentrations.

Acute administration of ethanol to normal men does not affect plasma T concentrations (52, 53, 54, 55, 56), whereas chronic administration ($\geq$200 g ethanol per day for up to 30 days) decreases plasma T (57, 58). The latter studies, however, did not include controls. As T levels may be suppressed by stress, dietary factors, and physical activity (59, 60, 61, 62), such factors should be considered. Moreover one does not administer these amounts of ethanol without affecting the liver (57).

In cross-sectional studies of twins and healthy elderly men (63, 64) alcohol consumption was not related to plasma T. Further, normal plasma T concentrations were observed in chronic alcoholics without cirrhosis, both while drinking alcohol and following alcohol withdrawal (44, 65, 66, 67, 68, 69). *Lindholm et al* (68) observed normal non-protein bound T concentrations in spite of increased SHBG concentrations; accordingly these patients had normal plasma LH concentrations (70). The increased SHBG concentrations were not significantly related to serum oestradiol concentrations (68).

Although this lack of association was taken as evidence against a relation between oestradiol and SHBG production (68), recent observations make a different interpretation possible. First, alcoholics without cirrhosis have increased oestrogen concentrations (68, 69, 71), although other studies have observed normal concentrations (44, 65, 69). Second, alcohol increases the rat hepatic aromatase activity (72), making an increase of hepatic oestrogens likely. Further, alcohol increases the level of hepatic oestrogen receptor and decreases the level of the hepatic androgen receptor (73, 74, 75, 76). These effects could lead to the increased SHBG concentrations of alcoholics (25, 68, 77). Due to the stronger binding of T than oestrogens to SHBG, such an increase of SHBG could further increase the action of oestrogens through an increase of the metabolic active oestradiol/T-ratio (78).

In summary, it still remains to be demonstrated conclusively that alcohol consumption *per se* reduces plasma T of men. However, chronic ethanol consumption may through its effect on oestrogen metabolism and on hepatic microsomal enzyme function (79) increase SHBG levels. This could increase the oestrogenic and decrease the androgenic activity, and lead to symptoms like gynecomastia seen in non-cirrhotic alcoholics (3).

Plasma testosterone concentrations in men with alcoholic cirrhosis

Plasma T concentrations have been reported to be low or normal in men with alcoholic cirrhosis (2, 3), and it has been considered that ethanol – not liver dysfunction – causes the low plasma T concentrations (2, 3, 47, 58, 80, 81). In contrast we found median plasma T concentrations in alcoholic cirrhotic men which did not differ significantly from those of normal controls or non-alcoholic, non-cirrhotic patients (35, 37, 39, 40, 41, 42) (Fig. 3). When T concentrations were related to liver function on a quantitative basis, we were unable to disclose any significant relations apart from a positive correlation with serum albumin ($r=0.44$, $p<0.01$) (36).

However, when the severity of liver cirrhosis was estimated by a modified Child-Turcotte's classification (37, 40), which is related to portal pressure and contains significant prognostic information regarding death (41, 82), we observed a significant association between decreasing liver function and declining plasma T concentrations (Fig. 4). The patient groups did not differ significantly regarding ethanol consumption, and the liver function-T association was still present when data on ethanol consumption were included in a multivariate analysis (40). Due to the raised SHBG levels (37, 40) patients with preserved (Group A) and moderately decreased (Group B) liver function had median plasma T levels above the controls (Fig. 4). Patients with severely decreased liver function (Group C) had lower plasma T than controls. The relation between liver function and plasma concentrations of T, non-protein bound T and non-SHBG bound T can also be observed from Fig. 5. As demonstrated, the majority of patients had plasma concentrations of non-protein bound T and non-SHBG bound T in the lower range of controls or depressed values. Neither in the first (37, *C. Gluud et al,* unpublished observations) nor in the second patient series (40) were we able to demonstrate any relation between ethanol consumption and plasma T concentrations.

In conclusion, decreasing liver function, but not ethanol consumption, seems to be responsible for the low levels of T in alcoholic cirrhotic men. Due to the increased SHBG levels, however, patients with preserved liver function may have supranormal plasma T concentrations. This is in accordance with observations by *Shlimovich* and *Kogan* (83) and *Zumoff et al* (84). Further, the increased SHBG levels lead to low concentrations of non-protein bound and non-SHBG bound T, which also declines with decreasing liver function.

Is the liver function-plasma testosterone relation accidental?

As the association between liver function and plasma T has been observed in cross-sectional studies, the correlation may be

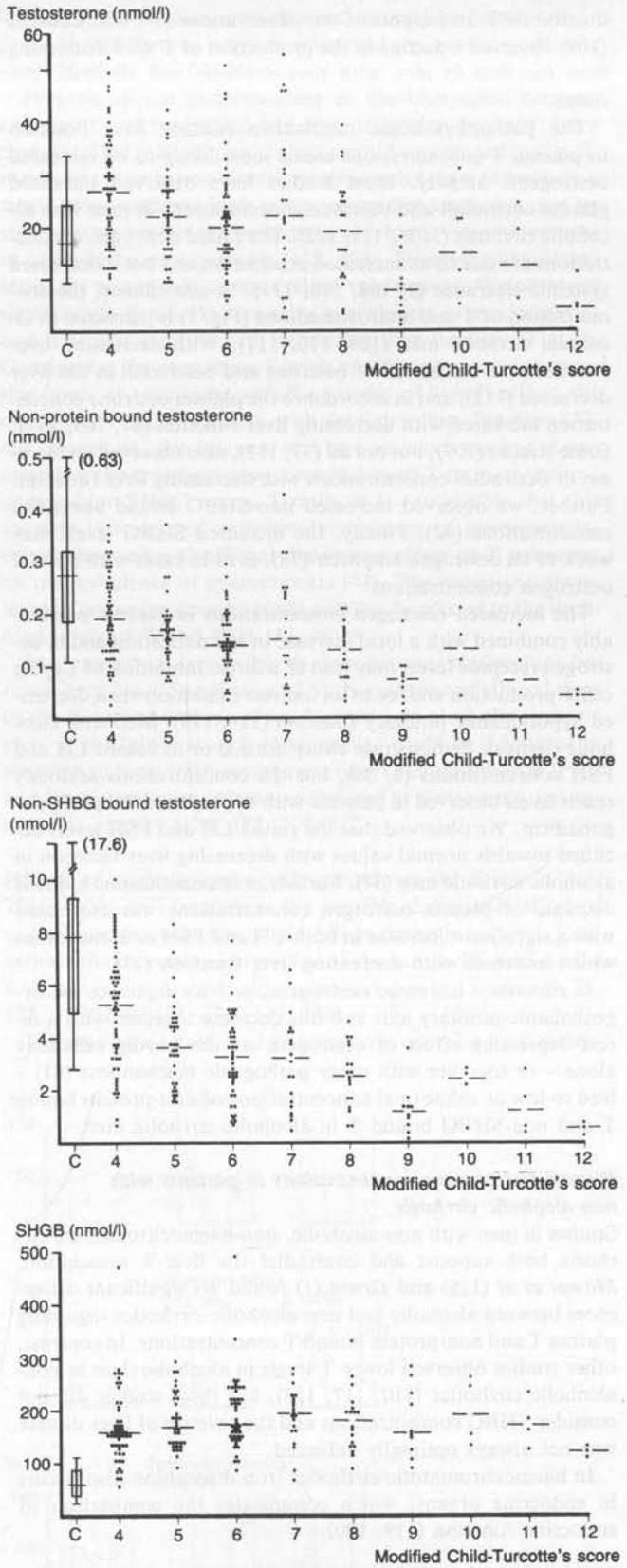


Fig. 5. *Plasma concentrations of testosterone, non-protein bound testosterone, non-SHBG bound testosterone, and SHBG in male controls (C) and in men with alcoholic cirrhosis. The patients are divided according to the modified Child-Turcotte's score. Increasing score means increasing severity of liver disease. Horizontal lines represent median values. In the control group, the vertical line represents the range and the box represents the interquartile range. Values obtained in individual patients are represented with a dot. Data from* Gluud et al *1987 (40). In patients, plasma concentrations of testosterone, non-protein bound testosterone and non-SHBG bound testosterone decreased significantly ($p < 0.001$, Kruskal-Wallis test) with increasing modified Child-Turcotte's score. SHBG concentrations were insignificantly related to the score.*

accidental. Factors influencing T levels could correlate with liver function and explain the association.

SHBG concentrations are significantly increased in men with alcoholic cirrhosis (3, 44) and this has been confirmed by us using DHT binding assays (37, 39, 40, 42). Binding assays correlate closely with RIA and electroimmunoassays (85, 86). It is assumed that the increased SHBG levels is due to increased oestrogenic and decreased androgenic actions on the liver leading to increased SHBG synthesis (25, 80, 87, 88, 89). However, a positive correlation between SHBG and T levels was observed (40) and SHBG is an unlikely explanation of the association between the modified Child-Turcotte's classification and plasma T. First, SHBG was included as a background variable (40). Second, SHBG concentrations are not significantly correlated to liver function (37, 40) (Fig. 5).

Stress is associated with inhibition of gonadotrophin secretion leading to low T concentrations (59, 90). However, stressed patients were excluded in the first series (37) and we were unable to disclose any significant differences among the patient groups with regard to cortisol and prolactin concentrations *(C. Gluud et al,* unpublished observations, 91). This makes stress a less likely explanation of the liver function-T relation.

Medication should be considered. A number of drugs used in cirrhotics may affect plasma T (14, 92, 93). In men with alcoholic cirrhosis the prevalence of spironolactone and loop-diuretic medication increased and sedative medication decreased significantly with declining liver function (Table 1) *(C. Gluud* and the Copenhagen Study Group for Liver Diseases (CSL), unpublished observations). No significant associations could be demonstrated between liver function and the prevalence of medication with thiazide diuretics, peroral antidiabetics, insulin, antihypertensive drugs, disulfiram, anti-epileptic drugs, vitamins and other drugs. For the following reasons, the liver function-plasma T relation does not seem to be due to differences in medication. Patients receiving spironolactone were excluded in our first patient series (37), and spironolactone medication was not significantly associated with plasma T in our second series (40). Moreover, spironolactone in doses at or below 100 mg per day (which was mostly used) is not always followed by a decrease in T levels (94, 95). Further, we were unable to disclose any association between loop-diuretics and plasma T levels when liver function was considered *(C. Gluud* and CSL, unpublished observation). Lastly, if sedatives were to affect plasma T, one should expect a decrease (14).

Concurrent diseases like diabetes mellitus, renal dysfunction, and hypothyreoidism should be considered (14, 96, 97, 98, 99). However, patients with concurrent diseases were excluded (37). In the second series (40), 9% of the patients were diabetics. However, the prevalence was not related to liver function, and none of the diabetics were examined during poor metabolic control. Well controlled diabetics have normal plasma T levels (96, 97). Serum creatinine was included as a background variable, and was not significantly associated with plasma T concentrations (40). Although patients in both series (37, 40) appeared to be euthyroid, we have demonstrated a positive correla-

Table 1. *Prevalence (with 95% confidence limits) of various medications in alcoholic cirrhotic men divided according to the modified Child-Turcotte's classification. Data are from patients included in ref. 41 and 172.*

	Group A (n = 46)	Group B (n = 50)	Group C (n = 14)
Spironolactone	17% (8-31%)	27% (39-68%)	79%*) (49-95%)
Loop-diuretics	15% (6-29%)	54% (39-68%)	86%*) (57-98%)
Sedatives	30% (18-46%)	0% (0-7%)	0%*) (0-23%)

*) $p < 0.001$ (χ^2 for trend).

tion between plasma triiodothyronine and T concentrations (100). However, this may be due to a covariation with the severity of liver dysfunction as these patients demonstrated normal thyroxine and thyrotrophin levels (100).

Malnutrition and *overweight* are associated with low T concentrations. Severe protein restriction, lack of trace elements (zinc and manganese) and vitamins (A, B, D and E) and obesity may lead to low T concentrations (98, 101, 102, 103). In the first series (37), patients received a hospital diet plus vitamin supplementation for at least two weeks. This also applies to the majority of patients included in the second series (40). Further, Broca's index was included as a background variable and proved insignificantly related to plasma T (40), and the three patient groups (A, B and C) did not differ significantly regarding Broca's index. Finally, when a modified Broca's index was formed by subtracting 6 kilogrammes (kg) from the weight of all patients with ascites in order to correct for this variable in calculating Broca's index, we observed a significant ($p<0.01$ for homogeneity) decrease in median modified Broca's index among the groups (Group A 1.04 kg/cm (range 0.70-1.54); Group B 0.99 kg/cm (0.67-2.04); Group C 0.92 kg/cm (0.54-1.25)), but this index was not significantly related to plasma T levels *(C. Gluud* and CSL, unpublished observations).

Moderate *physical excercise* increases T levels, while exhausting physical activity causes a decrease (60). Although physical activity was not taken into consideration, all patients in the first series were ambulatory in-patients (37) and duration of hospitalization was included as a background variable in the second series (40). It was demonstrated that duration of hospitalization was inversely correlated to plasma T concentrations (40). None of the patients investigated engaged in exhausting physical exercise.

Genetic factors may account for 26% of T level variation and 34% of non-protein bound T level variation according to *Meikle et al* (63). Such genetic effects were not considered in our studies and might be relevant (81). Although some studies have observed an increased prevalence of certain HLA-antigens in patients with alcoholic cirrhosis (104), other studies have been unable to disclose such an increase (105, 106). Therefore, HLA-types do not seem a plausible explanation of the difference between controls and alcoholic cirrhotics regarding plasma T variation. However, we are unable to exclude that HLA and other genes may have an influence on plasma T variation among the patients.

Increasing *age* is associated with declining plasma T concentrations (60, 107). However, age is an unlikely explanation of the liver function-T relation as age was included in the multiple regression analysis (40), and the patient groups did not differ significantly regarding age (37, 40).

In summary, possible confounders do not readily explain the observed relation between liver function and plasma T concentrations.

Pathophysiologic mechanisms explaining the liver function-plasma testosterone relation

The concept of the importance of declining liver function for the decreasing plasma T concentrations in alcoholic cirrhotic men is in accordance with some observations (69, 108, 109).

It has previously been observed that men with alcoholic cirrhosis have a decreased systemic clearance of T (3, 108, 110, 111), which may be explained by the raised SHBG concentrations (111). However, by oral administration of a dose of T which was able to produce plasma T levels above the SHBG binding capacity, we observed an association between the decreased liver function and plasma T levels following the load (38). Recent data support that both decreased liver function and increased SHBG levels cause the reduction of systemic clearance of T (112). Accordingly, the low non-protein bound and non-SHBG bound T levels are caused by a decreased production of T. In support of our observations (37, 40), *Lourens* (108) observed a decline in the production of T with decreasing liver function in men with non-alcoholic and alcoholic cirrhosis.

The pathophysiologic mechanism relating liver function to plasma T concentrations seems most likely to be increased oestrogenic activity. Most studies have observed increased plasma oestradiol and oestrone concentrations in men with alcoholic cirrhosis (3, 36, 111, 112). The raised oestrogen concentrations are due to an increased production and not a decreased systemic clearance (3, 108, 110, 111). In accordance, the aromatization of T and androstenedione (Fig. 1) is increased in alcoholic cirrhotic men (108, 110, 111). With decreasing liver function, the extraction of oestrone and oestradiol in the liver decreased (112), and in accordance the plasma oestrone concentration increased with decreasing liver function (37, 109, 113). Some studies (109), but not all (37, 113), also observed an increase of oestradiol concentrations with decreasing liver function. Further, we observed increased non-SHBG bound oestradiol concentrations (42). Finally, the increased SHBG levels may work as an oestrogen amplifier (78), even in cases with normal oestrogen concentrations.

The increased oestrogen concentrations in plasma presumably combined with a local increase in aromatization and in oestrogen receptor levels may lead to a direct inhibition of Leydig cell T production and/or to an indirect inhibition via a decreased hypothalamic-pituitary function (114, 115). Men with alcoholic cirrhosis demonstrate either normal or increased LH and FSH concentrations (3, 36), but the concentrations seldomly reach levels observed in patients with hypergonadotropic hypogonadism. We observed that the raised LH and FSH levels declined towards normal values with decreasing liver function in alcoholic cirrhotic men (37). Further, a dexamethasone induced decrease of plasma oestrogen concentrations was associated with a significant increase in both LH and FSH concentrations which increased with decreasing liver function (37).

In summary, increased oestrogenic activity depresses the hypothalamic-pituitary axis and this decrease together with a direct depressing effect of oestrogens on the Leydig cells may alone – or together with other pathogenic mechanisms (81) – lead to low or subnormal concentrations of non-protein bound T and non-SHBG bound T in alcoholic cirrhotic men.

Plasma testosterone concentrations in patients with non-alcoholic cirrhosis

Studies in men with non-alcoholic, non-haemochromatotic cirrhosis both support and contradict the liver-T association. *Mowat et al* (116) and *Green* (1) found no significant differences between alcoholic and non-alcoholic cirrhotics regarding plasma T and non-protein bound T concentrations. In contrast, other studies observed lower T levels in alcoholic than in non-alcoholic cirrhotics (110, 117, 118), but these studies did not consider SHBG concentrations and the severity of liver disease was not always optimally balanced.

In haemochromatotic cirrhotics iron disposition also occurs in endocrine organs, which complicates the comparison of endocrine function (119, 120).

Consequences of hypoandrogenization and hyperoestrogenization in alcoholic cirrhotic men

Previously, hypoandrogenization was considered responsible for the hypogonadism (encompassing testicular atrophy, hypo- and aspermia, sexual dysfunction, and reduction of body hair, beard growth and prostatic size) and hyperoestrogenization for the feminization (encompassing gynecomastia, female body habitus and escutcheon, and arterial spiders) frequently observed in alcoholic cirrhotic men (1, 2, 3). However, studies attempting to relate plasma T levels to hypogonadism and plasma oestrogen levels to feminization have mostly been negative (36,

44, 68, 110). Further, no apparent association between liver function and signs of hypogonadism and feminization could be established (1, 36, 44, 70).

Progress in the understanding of the interaction between androgens and oestrogens and their receptors, however, have demonstrated that relating plasma steroid concentrations to clinical signs may be too simple an approach (121). Moreover, a single cross-sectional look at e.g. oestradiol levels may not be sufficient to explain gynecomastia which may require months in order to become palpable (122, 123). However, when large series are considered an association may appear. We observed significantly ($p<0.05$) increasing prevalences of gynecomastia and testicular atrophy with decreasing liver function (Fig. 6). Considering the decreasing T levels and the increased or increasing levels of oestrogens with the severity of liver function, the oestrogen/T-ratio increases with declining liver function (37, 40). Moreover, this increase will be even more marked if one considers the oestrogen/non-protein bound T-ratio or the oestrogen/non-SHBG bound T-ratio. It is conceivable that this increase is associated with the development of gynecomastia, as we observed a significant depressing effect of T treatment on the prevalence of gynecomastia (41). The increasing prevalence of testicular atrophy could possibly be related to the declining levels of T and FSH (37, 40).

In summary, it is possible to demonstrate relationships between liver function, changes of sex hormones and clinical signs of the disturbed balance of sex hormones in alcoholic cirrhotic men. It is likely that such relationships could be deliniated in more detail from the measurement of sex steroid receptor levels and from circulating compounds related to oestrogenic and androgenic activity (124, 125, 126, 127).

Effects of anabolic-androgenic steroids on the liver

This section will summarize the possible therapeutic effect of anabolic-androgenic steroids (AAS) in cirrhotic patients. Concerning the increasing knowledge of the actions of AAS on the liver, the reader is referred to recent reviews and studies (9, 76, 128, 129, 130, 131, 132, 133, 134).

It seems now established that the liver contains both androgen receptors and oestrogen receptors (76, 135, 136), but certain effects of AAS on the liver (e.g. increase in rat liver weight) may use androgen receptor independent mechanisms (121, 137).

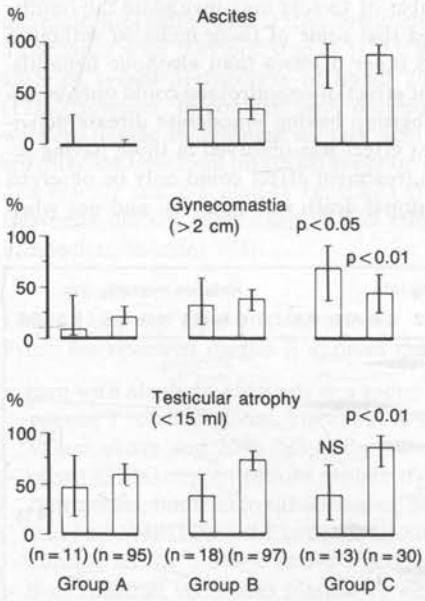


Fig. 6. *Prevalences (with 95% confidence limits) of ascites, gynecomastia (>2 cm in diameter on one or both sides), and testicular atrophy (mean volume <15 ml) in men with alcoholic cirrhosis divided into group A, B, and C according to the modified Child-Turcotte's classification. Data from* Gluud et al *1983 (37) (left) and* Gluud et al *1987 (40) (right). N are number of patients. NS denotes not significant. P-values are calculated by the χ^2-test for trend within each study. No p-values are calculated for the prevalence of ascites as this variable was included in the division of patients.*

Male sex may protect the liver against a number of noxious substances, including ethanol (9). Studies demonstrating a decreasing effect of AAS on lipid accumulation caused by such substances suggest that AAS may be responsible for the sex difference (9). Human studies have suggested an increased risk of alcoholic liver disease in women (138) and demonstrated a faster clearing of fatty change in male alcoholics following AAS (9). Moreover, T may reverse an ethanol induced inhibition of chick fetal hepatocyte growth (139) and animal studies have demonstrated an increased liver weight following AAS treatment (9), although some studies have been unable to demonstrate this effect (140). It remains to be demonstrated if AAS can increase liver DNA, but AAS may stimulate hepatocyte RNA and protein synthesis (9).

In a meta-analysis (141), the author (9) made probable that AAS treatment may reduce the relative mortality risk (RR) of cirrhotics to 0.46 (95% confidence limits 0.23-0.90, ($p=0.025$)). From the reviewed studies it appeared that AAS treatment had to be given for months in order to be effective (9).

Effect of oral testosterone treatment on plasma sex steroid concentrations

In a phase II study, we compared the effect of injection of T esthers intramuscularily to that of oral T administration on plasma T levels (35). Oral T was examined as it seemed convenient for long-term studies (142). Moreover, as an effect of T on the liver was supposed, the oral route enabled us to expose the liver directly to T. *Foss* (143) was the first who administered T by the oral route and he reported potency in a male castrate following 100 mg per day. Reports have demonstrated that oral T administration leads to absorption of T via the portal tract. Little is absorbed via the intestinal lymphatics (144, 145). Metabolites are mainly excreted via the kidneys, only small amounts appear in the bile (144, 145). The time course and amounts of radioactive metabolites in urine appear to be very similar following intravenous and oral administration of T, more than 90% of the urinary activity being excreted within 48 hours (146).

In spite of these observations it has become common belief that oral T administration is ineffective (147, 148). However, *Johnsen et al* (149) demonstrated that oral administration of micronized (<15 µm crystals) T is followed by a significant increase in plasma T concentrations, and an effect on sexual ability in men with testicular atrophy was observed. Even larger T cystals (125-400 µm) may be effective in raising plasma T concentrations after oral administration (150).

T propionate (100 mg intramuscularily every second day corresponding to 84 mg T) produced median concentrations of T of about 105 nmol/l in alcoholic cirrhotic men (35). When administering 200 mg of micronized T q.i.d. median T concentrations were about 175 nmol/l. Further, we noticed a larger variation in plasma T concentrations following oral than following parenteral administration (35). Therefore, the relation between plasma T concentrations two hours after an oral load of 400 mg T and liver function was examined (38). The increase of plasma T after the load (log T) correlated inversely with hepatic functions. These results were an extension of and in accordance with observations demonstrating higher plasma T concentrations in men with liver cirrhosis than in normal men following an oral dose of 63 mg T (151). However, our observations (38) were in contrast with those of *Nieschlag et al* (152) who demonstrated a greater increase of plasma T concentrations in compensated than in decompensated cirrhotics. The explanation for these differing results are not evident. *Nieschlag et al* (152)

used the presence of ascites to define patients with decompensated disease. When this criteria was used in our material (38), the median increase of T was 93 nmol/l (range 13-634 nmol/l, n = 25) in patients without ascites compared with 152 nmol/l (range 50-664 nmol/l; n = 17) in patients with ascites ($p < 0.05$) *(C. Gluud et al,* unpublished observations). In support, *Kley et al* (109) demonstrated higher plasma T concentrations with more severe decompensation following T enanthate injection.

Aromatizable AAS lead to a significant increase in plasma oestrogen concentrations, and the higher plasma T levels get, the higher will plasma oestradiol concentrations reach (109, 153, 154). Due to the large doses used for oral T treatment (35, 38, 142, 149), a significant increase in plasma oestradiol and oestrone concentrations could be anticipated. We therefore determined plasma and urinary concentrations of oestrogens in males with and without alcoholic cirrhosis following oral T administration (42). This study demonstrated that the increase of both oestrogens and androgens was significantly higher in the cirrhotics than in controls. Moreover, the cirrhotic group achieved urinary excretion of oestrogens which was significantly higher than in controls in whom no significant increase could be demonstrated (42). Further, when a sample of the patients included in the T trial (41) was examined before and following oral T treatment plasma concentrations of oestrone and oestronesulphate increased significantly, while only an insignificant increase in plasma oestradiol was observed (155).

In conclusion, oral administration of T leads to a significant increase in plasma T, an increase which rises with decreasing liver function. In alcoholic cirrhotic men, oral T increases the plasma oestrogen concentrations, but the oestrogen/T-ratio declines.

Effects of oral testosterone treatment of alcoholic cirrhotic men

Due to the lack of effective therapy of cirrhotics (156) and the possibility that AAS could improve the prognosis of cirrhotic patients (9), we started in 1979 enrolling men with alcoholic cirrhosis in the Copenhagen multicenter trial of oral T treatment versus placebo (41). Before this phase III study, phase II studies (35, 38) demonstrated no frequent side-effects. The patients entering the trial (41) had all histologically verified cirrhosis, 51% also demonstrating alcoholic hepatitis in the biopsy. The cirrhosis diagnosis was confirmed by liver vein catheterization and follow-up liver biopsies in a number of cases (82, 157, 158). The prevalence of anti-HBs antibodies in these patients (41) is in accordance with previous studies (159, 160), and does not seem to imply a viral ethiology of the cirrhosis.

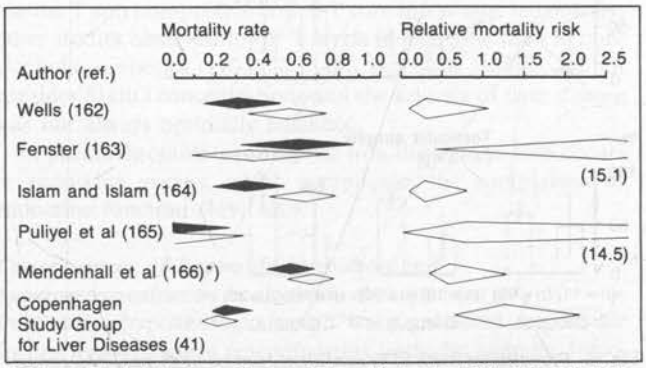
Taking significant prognostic indicators (group A, B and C of modified Child-Turcotte's classification and incapacitation index) and age into consideration, we found a RR of 1.17 (95% confidence limits: 0.65-2.15) comparing the T group to the placebo group (41). Similar results were obtained when more accurate prognostic indicators, such as portal pressure, were included in the analysis (82). Further, when only including the patients with histologically verified alcoholic hepatitis in the analysis, the RR was 1.10 (95% confidence limits: 0.50-2.39) comparing the T group to the placebo group (41). Accordingly, we are able to exclude with 95% confidence that oral T treatment reduces mortality more than 50%, which was the hypothesis tested in the trial (41).

In addition, oral T treatment did not significantly affect conventional liver biochemistry, complications to cirrhosis and causes of death (41) as well as the prevalence of alcoholic hepatitis, fatty change, and a number of other morphologic variables in follow-up liver specimens (158). Further, T treatment did not significantly affect the development of macronodular cirrhosis (or hyperplastic nodules) in micronodular cirrhosis either when examining all follow-up liver specimens (158), or only percutaneous follow-up liver biopsies (161). Finally, oral T was without significant effects on liver haemodynamics and liver functions in the group of patients studied (157).

Do anabolic-androgenic steroids improve the survival of cirrhotic patients? Fig. 7 shows the mortality rates and RR of controlled studies on AAS-treatment of cirrhotics (41, 162, 163, 164, 165, 166). Only one study (164) observed a significant improvement of survival by introducing AAS treatment. Further, *Mendenhall et al* (166) were able to demonstrate a significant improvement of the six-month conditional survival in a subgroup of oxandrolone treated patients.

Supposing that AAS treatment increases survival, what could be the explaination of the lack of a significant mortality reduction? A number of factors could either alone or in combination explain the negative results. First, all studies were dealing with small populations which entails a risk of type II error. Moreover, patients may not have been balanced regarding a number of prognostic variables at entry and during follow-up. Further, disease activity may be pertinent to the therapeutic outcome (156, 166). Second, the type of AAS should be considered. Aromatizable AAS could have an unpredictable effect due to the increase of oestrogen concentrations. In rats, oestrogens may inhibit the effects of AAS on the liver (128, 167). Third, a number of patients in the Copenhagen trial (41) were treated with spironolactone (Table 1), which may decrease hepatic androgen receptor levels and interact with T binding to these receptors (168). However, we were unable to demonstrate any significant interaction between the severity of liver disease, which correlated to some measure with spironolactone medication (Table 1), and treatment effect (41). Fourth, recent studies suggest that the human cirrhotic liver contains less androgen receptors than normal liver (135, 136, 169). Therefore, differences in the receptor levels could possibly explain the differing therapeutic outcomes (Fig. 7).

AAS treatment does not improve survival of cirrhotic patients. The studies observing a beneficial effect of AAS on survival may also be subjected to criticism. *Islam & Islam's* study (164) was unblinded, which may lead to bias. Further, 26% of the patients were lost for follow-up. In the study by *Mendenhall et al* (166) a number of factors may invalidate the results. It can not be excluded that some of those included without a biopsy may have had other diseases than alcoholic hepatitis. Second, the significant effect of oxandrolone could only be demonstrated in the subgroup having »moderate disease activity«, and no significant effect was observed in those having severe disease. Third, a treatment effect could only be observed in a six-month conditional death rate analysis, and not when



*) This study included patients with alcoholic hepatitis, but about 70% of the patients demonstrated liver cirrhosis as well.

◆ AAS group
◇ Control group

Fig. 7. *Mortality rates and relative mortality risks (with 95% confidence limits) in cirrhotic patients treated with anabolic-androgenic steroids (AAS group) or receiving no treatment or placebo (control group).*

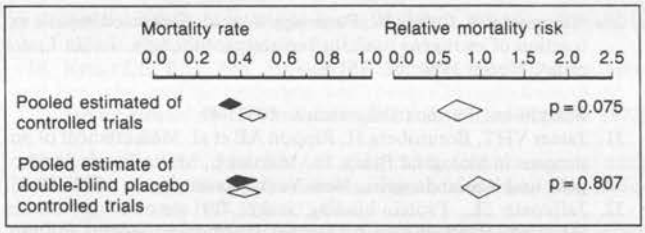


Fig. 8. Pooled estimates of mortality rates and relative mortality risks in controlled trials (n = 6 from Fig. 7) and double-blind, placebo controlled trials (Fenster, *1964 (163),* Mendenhall et al *1984 (166), and* the Copenhagen Study Group for Liver Diseases, *1986 (41)) including cirrhotics treated with anabolic-androgenic steroids (AAS). P-values are calculated by the Mantel-Haenszel test.*

using Kaplan-Maier life-table analysis. This entails a risk of mass significance. Moreover, the conditional analysis only includes patients who have survived the acute phase of the disease. Therefore, treatment groups may no longer be comparable.

Accordingly, it still remains to be demonstrated that AAS may beneficially affect the course of patients with cirrhosis and alcoholic hepatitis. As previously outlined (9), a meta-analysis of the published results may be misleading due to the differences in patients included, drugs, dose, duration of treatment and follow-up. However, when a pooled estimate of the RR of the studies of Fig. 7 is calculated (Fig. 8) no significant difference appears[1]. This is also the case when only the three double-blind, placebo controlled studies are included[2] (41, 163, 166). Accordingly, AAS do not seem to reduce mortality by more than 23%, but may also increase mortality by 22%. Therefore, the therapeutic gain regarding survival is small, if it exists, and side-effects like thrombosis can not be excluded (41, 158).

Effect of oral testosterone treatment on well being and sexual function in alcoholic cirrhotic men

Men with alcoholic cirrhosis suffer from continued alcoholism, increased morbidity, and sexual dysfunction (41, 158, 170). In accordance with previous studies (171), we observed a prevalence of sexual dysfunction of about 70% in these patients (172). However, in contrast with previous observations (171) we were unable to demonstrate any significant effects of oral testosterone treatment on sexual function (encompassing libido and erectile and ejaculatory function) (172). Further, oral T treatment did not significantly affect ethanol consumption or incapacitation index (41).

Conclusions and final considerations

From the reviewed studies it appears that

- men with alcoholic cirrhosis as a group have normal median plasma T concentrations, but about 20% of the patients have values above and 20% below the normal limits,
- raised SHBG concentrations explain the supranormal T concentrations, but lead to subnormal or low non-protein bound and non-SHBG bound T concentrations, which are currently considered the biologic active fractions,
- liver function influences plasma T, which declines with decreasing liver function, and a concomittant increase of the prevalence of gynecomastia and testicular atrophy was observed,
- ethanol may not be the central factor in producing low T levels in alcoholic patients, and further studies using adequate controls are needed to establish the effect of ethanol on T in men.

Furthermore, in order to understand the combined mechanisms of hypoandrogenization and hyperoestrogenization in more detail, studies should examine the effects of e.g. anti-oestrogenic drugs and the removal of oestrogens. Finally, the interaction between sex steroids and their receptors needs investigation.

Based on therapeutic studies, oral testosterone treatment

- significantly increases plasma T concentrations, an increase which is accentuated with decreasing liver function. Further, cirrhotic patients obtain higher oestrogen concentrations than controls. The latter increase may have implications for the lack of effect and possible side-effects.
- does not significantly influence survival, liver functions or morphology,
- and does not significantly improve well being and sexual function of alcoholic cirrhotic men.

SUMMARY

The present review summarizes the pathogenic mechanisms leading to variation of plasma testosterone concentrations, consequences of hypoandrogenization and hyperoestrogenization, and effects of oral testosterone treatment in men with alcoholic cirrhosis.

These patients have normal median plasma testosterone concentrations, but 20% have values above and 20% have values below the normal limits. The majority of patients have raised sex hormone binding globulin (SHBG) concentrations. This increase accounts for the supranormal plasma testosterone concentrations. With decreasing liver function, plasma testosterone concentrations decrease significantly. The combination of increased SHBG levels and decreasing liver function leads to low or subnormal plasma concentrations of non-protein bound and non-SHBG bound testosterone. This decrease, together with raised oestrogen concentrations, may explain the increased prevalence of gynecomastia and testicular atrophy which raises with decreasing liver function.

Oral testosterone treatment of alcoholic cirrhotic men produces an increase in the plasma concentrations of testosterone, androstenedione and dihydrotestosterone, but oestrogen concentrations increase as well. Oral testosterone treatment significantly reduces the prevalence of gynecomastia, but is without significant effects on liver biochemistry, morphology, haemodynamics, and function, general well being, sexual dysfunction and survival of alcoholic cirrhotic men.

A pooled estimate of the mortality risk of cirrhotic patients treated with anabolic-androgenic steroids does not disclose any significant difference compared with placebo treatment (relative risk 0.98; 95% confidence limits 0.77-1.22). Seldom, but serious, side-effects of oral testosterone treatment can not be excluded.

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1) RR = 0.73; 95% confidence limits 0.51-1.03.
2) RR = 0.98; 95% confidence limits 0.77-1.22.

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The concentration of ionised calcium in plasma

Measurement and some clinical applications

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INTRODUCTION

Calcium ions in plasma are either free (ionised) or bound to protein and small anions. The most important calcium binding protein is albumin, and the most important calcium binding anions are bicarbonate, citrate, phosphate, lactate, and sulphate. Only the ionised calcium is physiologically active and regulated by parathyroid hormone, $1,25(OH)_2$-D vitamin and calcitonin.

Almost 20 years ago a calcium electrode for routine measurement was developed, and it is hoped that this review will stimulate further research with the calcium electrode, which has since been improved. The binding of calcium ions in plasma depends on pH, which necessitates that the pH be controlled. pH must either equal pH in circulating plasma, or pH and ionised calcium must be measured together and ionised calcium subsequently corrected. If the original pH of the patient is known, ionised calcium may be corrected to this pH value. Ionised calcium at pH 7.40 is also useful even in a patient with an acute acid-base disturbance, where it will resemble the value before and after the acid-base disturbance. One of the purposes is to describe how ionised calcium depends on pH.

Some authors have suggested that potentiometry of ionised calcium is influenced by protein in the sample. We made some experiments in which ionised calcium depends on the protein concentration, but the observations were best explained as a Donnan distribution across a semipermeable membrane.

Clinically testing the method was another purpose. Pregnancy is associated with a stressed calcium homeostasis, because the mother delivers 20-30 g calcium to the fetus. We examined whether ionised calcium depends on gestational age in pregnant women. We measured calcium fractions in serum from morbidly obese subjects and insulin dependent diabetics. Calcium may be associated with essential hypertension, so we also examined ionised calcium and blood pressure in a group of 45-year olds.

CHEMICAL POTENTIAL

A component in a system can be characterized by its total (stoichiometric) concentration (amount of substance per unit volume) or its chemical potential (chemical free energy per amount of substance unit). An energetic point of view is not unusual to physicians. We measure and report the partial pressures of O_2 and CO_2, proportional to the activities, H^+ is measured and reported in pH units, proportional to minus the chemical potential of H^+, and osmolality is proportional to minus the chemical potential of water. In the human organism the free chemical energy of food is transformed to heat, work of muscle, nerve conductance, growth, repair, and maintenance of a constant internal environment. The total chemical free energy of the organism always decreases, and more so than the effective work. This law determines the direction of the net processes.

To studies of balance or metabolism the total concentrations and amounts of substance may be relevant, but otherwise the chemical potential is the relevant physiological quantity, because it determines the direction of the chemical processes, transport, binding of hormones to receptors etc.

The free energy or Gibbs energy is a function of state, which describes the maximum content of work of a system. It is assigned "G". $G = U + PV - TS$, and for a closed system with constant pressure P and temperature T, $\Delta G = \Delta U + P\Delta V - T\Delta S$. U is the internal energy of the system, V is the volume, and S is the entropy. $U + PV$ is the enthalpy H. The change in free energy ΔG of a chemical reaction can be defined as the free energy of the reaction products minus the free energy of the reactants. When ΔG is zero no net work can be obtained, and the reaction is in equilibrium. When ΔG is positive work must be expended to make the reaction take place. When ΔG is negative the reaction may occur freely, expending its own energy, and it is possible to gain work. The more work which can be gained, the farther is the reaction from equilibrium. The decrease in free energy is sometimes called the driving force of the reaction.

The chemical potential μ_i of an uncharged component in a system can be defined as the change in free energy of the system per unit component added at constant T and P. The amount of component added must be infinitesimal, and the composition of the system must otherwise be constant. An ion with charge z will add some electrostatic energy, $zF\Phi$, where zF is the charge per mole, and Φ is the inner potential, which is not measurable. It is not possible to experimentally separate the chemical potential of the ion from its combined electrochemical potential $\tilde{\mu}_i = \mu_i + zF\Phi$. However, the chemical potential of a neutral salt can be measured when positive and negative charges are added in equal amounts. With a non-thermodynamical convention the chemical potential of single ions can then be defined from the chemical potential of the salt.

No natural zero point exists for the chemical free energy, and an arbitrary zero is therefore defined for G, H, and S of the pure elements at some standard state. By measurement, use of tables and calculation values can be obtained for the chemical free energy of all substances in the standard state.

ACTIVITY

The thermodynamic functions of ideal systems can be generalized to include non-ideal systems. In an ideal solution the chemical potential of a component $\mu_i = \mu_i$ (pure component) $+ RT \ln x_i$, where R is the gas constant, T is the absolute temperature, and x_i is the mole fraction. The similar generalized expression is $\mu_i = \mu_i^\circ + RT \ln a_i$ with the chemical standard potential μ_i° and the activity a_i of unit 1 (one). The values of μ_i° and a_i depend on the choice of scale (concentration, molality, mole fraction etc.) since the values of $a_i(c)$, $a_i(m)$, $a_i(x)$ etc. must infinitely approach those of c_i, m_i, x_i etc. upon infinite dilution. In a non-ideal solution the standard state is hypothetical with $a_i = 1$ on the chosen scale. In some systems simple expressions can be obtained for activity coefficients (y_i, γ_i, f_i etc.), which change the stoichiometric measure into activity ($a_i(c) = y_i c_i$, $a_i(m) = \gamma_i m_i$, $a_i(x) = f_i x_i$ etc.). Since chemical standard potentials are known and have been tabulated