Plasma oestrogens in men with chronic liver disease

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SUMMARY A highly specific radioimmunoassay was used to measure the total plasma concentrations of the three principal unconjugated oestrogens: oestrone E₁, oestradiol E₂, and oestriol E₃ in normal males and in 21 males with various forms of chronic liver disease. In addition, the unbound concentration of plasma E₂ was established in the same group. About half of the patients with liver disease had overt feminising changes. Total and unbound plasma E₂ concentrations were within the normal range in all patients. Total plasma E₁ was significantly elevated only in those patients with liver disease and gynaecomastia, and a similar trend was seen for total plasma E₃.

Feminisation of some males with chronic liver disease has long been recognized (Corda, 1925). Disturbed metabolism of oestrogens has been postulated as the cause of these changes (Glass *et al.*, 1940; Engel, 1944; Rakoff *et al.*, 1944; Schiller and Pincus, 1944; Gilder and Hoagland, 1946). Measurements of unconjugated plasma oestrogens in these patients have been largely confined to oestradiol (E₂) but results have been conflicting (Korenman *et al.*, 1969; Cedard *et al.*, 1970; Chopra *et al.*, 1973; Galvao-Teles *et al.*, 1973; Kent *et al.*, 1973; Lourens, 1973; Adlercreutz, 1974; Van Thiel, *et al.*, 1974).

An extension of the study to include the other two major oestrogens, oestrone (E₁) and oestriol (E₃), appeared logical in this complex situation. We have therefore studied, for the first time, the basal total plasma concentrations of unconjugated E₁, E₂, and E₃ in the same plasma sample; in addition, we estimated the unbound concentration of E₂. Observations were made in 21 patients with various types of chronic liver disease and in a group of normal controls.

Methods

PATIENTS

Twenty-one male patients (mean age 46.9 years, range 27-59 years) were studied. Three had haemochromatosis, five had alcoholic fatty livers, nine had alcoholic cirrhosis, and four non-alcoholic cirrhosis.

One patient in each of the first two groups had gynaecomastia defined as palpable glandular tissue of > 1 cm in diameter; seven of the nine with alcoholic cirrhosis had gynaecomastia as did three of the four with non-alcoholic cirrhosis.

Diagnosis was established on the basis of liver biopsy in 20 patients. In the remaining patient, whose clotting defects prohibited liver biopsy, diagnosis was based on clinical history, physical findings, and laboratory tests (including radiology).

Venous blood was withdrawn into heparinised tubes between 9 and 10 am and plasma was immediately separated and stored at -20°C until assayed.

ASSAYS

Plasma samples (2 ml) were thawed, equilibrated with an internal standard for procedural losses, and extracted twice with five volumes of diethyl ether. The extract was then subjected to Abraham's system of Celite column chromatography (Abraham *et al.*, 1970) by discontinuous solvent elution using increasing concentrations of ethyl acetate in iso-octane. This yielded three separate fractions for each sample. Fractions thus obtained were resuspended in phosphate buffer and subjected to radioimmunoassay. Antibody raised to a conjugate of the 6-keto derivative of the parent steroid was used in each case. Cross-reactivity studies showed no steroid tested had a higher cross-reactivity than 5% (except the 6-keto derivative). Interassay variation was within 15% for all three assays.

Unbound (free) plasma E₂ was estimated by multiplication of total plasma E₂ (estimated as above) by its unbound fraction as determined by steady-state gel filtration (Anderson *et al.*, 1972; Fisher *et al.*, 1974). This method maintains physio-

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Received for publication 26 February 1976

logical conditions of temperature and pH and is performed on undiluted plasma.

Results

Basal total plasma E_1 concentrations are shown in Fig. 1. The normal controls had a mean plasma E_1 of 66.4 ± 3.8 pg/ml (mean $\pm$ 1 SEM). Patients with chronic liver disease were divided into two groups on the basis of the presence or absence of gynaecomastia. Those without gynaecomastia had a mean plasma E_1 of 69.8 ± 3.5 pg/ml, which was not significantly different from normal. Those with gynaecomastia had a mean plasma E_1 of 117.7 ± 13.4 pg/ml, which is both significantly higher than normal controls ($P < 0.005$)¹ and patients without gynaecomastia ($P < 0.05$)¹. Observations on patients with

¹Median test and Mann Whitney u statistic.

Fig. 1 Basal total plasma oestrone (E_1) concentrations in normal men and in liver disease patients with and without gynaecomastia. Brace: mean $\pm$ 1 SEM. ● = non-alcoholic aetiology. ▲ = alcoholic aetiology.

alcoholic liver disease appeared evenly scattered throughout both groups.

Total basal plasma oestradiol (E_2) concentrations are shown in Fig. 2. The mean total plasma E_2 for normal controls was 29.7 ± 2.3 pg/ml. Patients with liver disease were divided as before and those without gynaecomastia had a mean total plasma E_2 of 30.3 ± 2.8 pg/ml, while the mean value for patients with gynaecomastia was 33.5 ± 3.9 . There was no significant difference between these groups and normal controls and again observations on those patients with cirrhosis of an alcoholic aetiology were evenly scattered throughout both groups.

Unbound plasma E_2 concentrations are shown in Fig. 3. All observations except one fall within the normal range, and there are no significant differences between patients with or without gynaecomastia. Observations on patients with alcoholic liver disease were once again evenly distributed throughout both groups.

Total plasma oestriol (E_3) concentrations are shown in Fig. 4. The mean plasma E_3 of patients with gynaecomastia (50.2 ± 5.2 pg/ml) was sig-

Fig. 2 Basal total plasma oestradiol (E_2) concentrations in normal men and in liver disease patients with and without gynaecomastia. (Key as in Fig. 1).

Fig. 3 Unbound (free) plasma oestradiol (E_2) concentrations in liver disease patients with and without gynaecomastia compared with the normal range. (Key as in Fig. 1).

nificantly elevated ($P < 0.05$) above normal controls (36.8 ± 3.6 pg/ml), while patients without gynaecomastia (mean 41.9 ± 3.96 pg/ml) appeared to form an intermediate group which was not significantly different from either normal controls or patients with gynaecomastia.

Discussion

The occurrence of testicular atrophy and gynaecomastia in some males with chronic liver disease has long been recognized, but the endocrine basis of such changes remains to be established. The conventional hypothesis, first proposed by Glass *et al.* (Glass *et al.*, 1940) is that the damaged liver fails to inactivate the endogenous oestrogens which are known in normal males to derive both from peripheral conversion of androgens to oestrogens (Longcope *et al.*, 1969) and also from direct testicular secretion (Kelch *et al.*, 1972). The hyperoestrogenic state thus induced in

Fig. 4 Basal total plasma oestriol (E_3) concentrations in normal men and in liver disease patients with and without gynaecomastia. (Key as in Fig. 1).

cirrhotic males would then explain both the hypogonadism (by direct suppression of pituitary gonadotrophins) and also the feminisation.

Many studies have recently confirmed the hypogonadism by demonstrating reduced circulating unbound plasma testosterone concentrations and oligospermia or azospermia in cirrhotic males (Chopra *et al.*, 1973; Galvao-Teles *et al.*, 1973; Mowat *et al.*, 1976), but studies on oestrogens and their metabolites are less conclusive (Adlercreutz, 1974). The early finding of increased urinary oestrogens during the course of acute liver disease using biological assay methods (Gilder and Hoagland, 1946; Llamasa and Gomez Mont, 1953) was not confirmed when biochemical methods of measurement were used (Gregoris, 1957; Müller, 1958). More recently, the measurement of plasma oestrogens (by competitive-binding or immunoassay) has been applied. These studies have been largely confined to the most potent oestrogen, oestradiol (E_2),

but results have also been conflicting (Korenman *et al.*, 1969; Cedard *et al.*, 1970; Chopra *et al.*, 1973; Galvao-Teles *et al.*, 1973; Kent *et al.*, 1973; Lourens, 1973; van Thiel *et al.*, 1974) with the majority of studies reporting normal total plasma E_2 concentrations in cirrhotic males.

In this complex situation, it seemed possible that there might be deranged metabolism of oestrogen metabolites other than E_2 in cirrhotic males which might contribute to the observed endocrine changes. This study was therefore designed to measure the three principal unconjugated oestrogens normally present in male plasma, oestrone (E_1), oestradiol (E_2), and oestriol (E_3). E_1 is the immediate precursor of E_2 , while E_3 is the major degradation product of the oestrogens in normal males, being excreted mostly as the glucuronide or sulphate conjugate.

The basal total and unbound plasma E_2 concentrations in our 21 patients with liver disease of varying aetiologies do not differ significantly from our normal range, regardless of the presence or absence of gynaecomastia. This is in agreement with a previous collaborative study of ours (Galvao-Teles *et al.*, 1973) in which E_2 concentrations were measured by protein-binding rather than radio-immunoassay. It is of interest that unbound E_2 concentrations are normal despite marked changes in steroid binding proteins associated with chronic liver disease.

Our new data concern the other two main oestrogens, E_1 and E_3 . There are two recent reports of plasma E_1 concentrations in patients with liver disease. Kley *et al.* (1975) found that plasma E_1 was significantly raised in patients with liver disease regardless of aetiology or the presence or absence of gynaecomastia. Van Thiel *et al.* (1975) report a similar rise but in their patients, all of whom had alcoholic liver disease, those with gynaecomastia had significantly higher plasma E_1 than those without gynaecomastia. Our findings confirm that there is a significant rise of plasma E_1 concentrations in patients with gynaecomastia when compared with normal males and with patients with no gynaecomastia. This is true irrespective of the aetiology of the liver disease. Although plasma E_1 tends to rise with age after 60 years (Kley *et al.*, 1974), all our patients were younger than this, making it unlikely that this factor contributed significantly to the increase. Furthermore, there was no significant age difference between the patients with or without gynaecomastia.

The elevated plasma E_1 concentration must be due either to increased formation of E_1 or to a decreased elimination or both. Thijssen *et al.* (1971) in a preliminary report, found elevated plasma androstenedione in men with cirrhosis of the liver. This could be a source for elevated plasma E_1 .

Impaired elimination of E_1 is also a possibility, although the tracer studies of Zumoff *et al.* (1968) have shown that conversion of E_1 to 16α -hydroxy-oestrone (the first step in the major degradative pathway) is not impaired in cirrhotic patients. The contribution of elevated plasma E_1 to feminisation is difficult to assess. A direct feminising action seems unlikely as E_1 is a biologically weak oestrogen (Vermeulen and Verdonck, 1968). An indirect action—for instance, at hypothalamic-pituitary level—is another possibility. Pituitary function tests performed in these patients have suggested that, in feminised patients only, there may be a circulating inhibitor of gonadotrophin releasing hormone (Mowat *et al.*, 1976). It might be that E_1 acts in this way.

We have also found elevated plasma E_3 concentrations in patients with gynaecomastia. The only other recent study on this plasma steroid in liver disease (Pentikäinen *et al.*, 1975) reports that plasma E_3 was raised in men with liver disease, but found no correlation with gynaecomastia in this exclusively alcoholic series. Conversion of E_1 to E_3 via 16α -hydroxyoestrone is known to be relatively impaired in patients with cholestasis but whose liver function is otherwise normal (Adlercreutz *et al.*, 1974). This effect is attributed to the interruption of the entero-hepatic circulation in cholestasis, since Breuer and Breuer (1973) showed *in vitro* that the ability of liver microsomes to metabolise E_1 to E_3 was not impaired in cirrhotic livers. Impaired conjugation of E_3 is an unlikely explanation for our findings, as oestrogen conjugation has been shown to remain unimpaired even in advanced hepatic failure (Adlercreutz and Tenhunen, 1970). It may be that our finding of elevated levels merely reflects a normal rate of degradation of the elevated E_1 . It is uncertain how much oestrogenic activity E_3 has, and its importance in causing feminisation is uncertain.

In conclusion, we have found raised plasma E_1 and E_3 concentrations in association with normal total and unbound plasma E_2 concentrations in the men with liver disease and gynaecomastia, and this suggests that the increase of one or both of these oestrogens may be of aetiological importance in causing feminisation in these patients. There is no association between these abnormalities of oestrogen metabolism with any aetiological factor, notably alcohol.

We are grateful to the Wellcome Research Foundation and the North East Thames Area Health Authority for financial support. We are also grateful to Professor J. Landon for the use of his laboratory and to Miss Monica Leighton for help with the statistical analyses of results. We are also grateful

to the following for their provision of the antibodies for the assays: Dr A. Boulton for E₁ antiserum, Dr B. Furr for the E₂ antiserum, and Miss Shahla Khoshroo for the E₃ antiserum.

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