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CLUES TO UNDERSTANDING THE OXIDATION OF ESTRADIOL IN HUMANS: EFFECTS OF ACUTE INFECTIOUS HEPATITIS, AUTOIMMUNE HEPATITIS AND CHRONIC LIVER DISEASE

Robert G. Lahita¹, Robert A. Schaefer³, H. Leon Bradlow⁴, and Mary Jeanne Kreek²

¹ Jersey City Medical Center, New York, NY 10021

² The Rockefeller University, New York, NY 10021

³ The New York Hospital-Cornell Medical Center, New York, NY 10021

⁴ Jurist Institute for Research Hackensack University Medical Center, Hackensack, NJ 07601

Abstract

Determination of 2- and 16 α -hydroxylation of estradiol in patients with a variety of liver disorders using a dynamic method of quantitating the extent of hydroxylation, revealed specific and characteristic differences in the metabolic response. Patients with acute or silent variants of hepatitis B had estrogen metabolite patterns that were indistinguishable from those found in the control subjects. Female patients with Autoimmune hepatitis (formerly known as Lupoid hepatitis), however, showed a moderate significant decrease ($p < 0.01$) in 2-hydroxylation as compared with normal controls (mean 16.3 ± 1.9 vs 33.9 ± 2.5), with no significant change in 16 α -hydroxylation. Male and female subjects with chronic alcoholic cirrhosis were almost devoid of 2-hydroxylation (mean 2.9 ± 0.5 , $p < 0.01$), but did show a significant increase in 16 α -hydroxylation ($p < 0.01$). The results, therefore, show that the alterations in patterns of biological oxidation are highly specific and do not reflect a general inability to metabolize estrogens in the cirrhotic patient. However, the results also suggest the possibility that a substantial fraction of 16 α -hydroxylation may occur elsewhere in the body at sites other than in the liver, explaining why this biotransformation pathway is elevated, while the reaction at C-2 is almost absent in the alcoholic cirrhotic subjects.

Keywords

liver disease; estrogen metabolism; alterations

Introduction

The occurrence of feminization and increased levels of estrogens in the setting of severe chronic liver disease is well established. The association of altered estrogen with disposition to hypogonadism, cholestasis, and hepatocellular carcinoma has been reported in this setting (1–17). Moreover, in diseases like systemic lupus erythematosus and in patients with breast cancer, elevated levels of 16-hydroxylation have been reported. Moreover, recent data indicate that hydroxylation of estrogens in the synovium of rheumatoid arthritis patients is also elevated in the 16 direction. These are some of the reasons why there is need to study

the effects of chronic liver disease on the dynamics of estrogen metabolism, and to determine whether enhanced production of metabolites with greater estrogenicity, such as 16 α -hydroxyestrone, and decreased formation of metabolites which are only weakly estrogenic, or even antiestrogenic, such as 2--hydroxyestrone and 2-methoxyestrone, the so-called "catechol" metabolites, occur. Although the hormonal relationships between the connective tissue diseases like lupus and rheumatoid arthritis and liver disease are unclear, there is need to provide these data for future research.

A highly sensitive and specific radiometric method for measuring biological hydroxylation of estradiol in humans was developed in our laboratories (18–23). This technique allows the assessment of the biotransformation of estradiol to 2- and 16 α -hydroxylated metabolites of estrone, and has been validated in studies of normal males, pre-menopausal and postmenopausal females (18), obese subjects (19), and patients with systemic lupus erythematosus (20).

In this study, these techniques are used to determine the extent of 2- and 16 α -hydroxylation of estradiol in patients with a variety of defined hepatic disorders, which include acute and subacute hepatitis B infection, the "silent" hepatitis B antigen carrier state, non-A and non-B type biopsy-documented chronic, aggressive, or active hepatitis, autoimmune hepatitis of the so-called "Lupoid" type, and finally, subjects with chronic alcoholic cirrhosis. The hypothesis tested in this study is that severe chronic liver disease, specifically cirrhosis, may alter estradiol metabolism, which results in the formation of increased amounts of the highly estrogenic 16 α -hydroxylated metabolite of estradiol and a reduction in the amounts of the weakly estrogenic or catechol estrogens.

Material and Methods

Normal healthy, volunteer subjects, recruited by advertisements in neighboring institutions, were selected who had no evidence of any significant disease and normal results on screening biological tests. Control subjects were required to be in a normal weight range and eating a standard diet. Women taking oral contraceptives and men or women receiving sex hormone therapy of any type were excluded from study. All women of child-bearing potential received a pregnancy test within 48 hours prior to the study and were studied only if not pregnant.

Nineteen patients (9 males and 10 females) with clinical, biochemical, and, in most cases, histological documentation (liver biopsies performed in 15 of 19 subjects) of acute, subacute, or chronic liver disease, or serological documentation of the chronic hepatitis B carrier state, were studied.

None of the study subjects had evidence of recent parenteral narcotic or other recent drug or alcohol abuse. All six chronic alcoholic subjects had a history of prior alcohol abuse for many years, but none had used alcohol in the past three months or longer; a past, but not current history of drug abuse had been present in several patients in each of the groups. Both the patients with liver disease and the control subjects ranged in age from 40 to 62 years.

All study subjects, including the patients with liver disease, the silent carriers of hepatitis B antigen, and the healthy control subjects, were hospitalized in the GCRC of The Rockefeller University Hospital for at least one day prior to the study. Extensive medical histories, complete physical examinations, and a panel of routine laboratory tests was performed in all study subjects to rule out the presence of any other disorder known to alter estrogen metabolism.

Written and oral informed consents were obtained from each patient prior to initiation of these studies. The research protocols and procedures were reviewed and approved by the Rockefeller University Human Research Committee (IRB) and by the Subcommittee on Use of Radioisotopes in Human Research Subjects.

The group of study subjects with liver disease included: 3 patients with acute and subacute viral (B type) hepatitis (Group 1); 2 “silent” chronic carriers of hepatitis B virus defined as persistent (hepatitis B antigenemia for more than six months with no clinical symptoms) (Group 2); 3 patients with non-A, non-B viral type chronic active hepatitis, all with liver biopsies confirming diagnosis (Group 3); and 5 female patients with autoimmune hepatitis type I characterized by hypergammaglobulinemia and positive antinuclear antibodies (Group 4). All had liver biopsies with both histories and serological profiles confirming diagnosis. Finally there were 6 patients with alcoholic cirrhosis, 5 with confirmatory liver biopsies (Group 5). The clinical laboratory and biopsy data for each of these patients are summarized in Table 1.

Quantitative radiometric determinations of the biological oxidation of [$16\alpha\text{H}^3$]-estradiol to determine the normal extent of 16α -hydroxylation were carried out in 27 normal, healthy control females and 30 normal, healthy control males, and the extent of 2-hydroxylation was determined in 21 females and 28 males (healthy, control subjects) using [2H^3]-estradiol as substrate. All 19 study subjects with liver disease underwent studies of 2-hydroxylation; 17 underwent studies of 16-hydroxylation.

The labeled estrogens used in these studies were specifically synthesized at The Rockefeller University to place the tritium atom at metabolically active sites on the estradiol molecule, specifically at C-2 and C- 16α (18) (see Figure 1). The labeled estrogen was dissolved in sterile propylene glycol, sealed in single dose glass vials, and stored in a freezer at -20°C until used. Bacterial and pyrogen testing was performed on contents of vials from each batch of isotopically labeled material.

All patients were sequentially studied, first with the 16α -tritiated and then with the 2-tritiated estradiol. On the morning of day one, each subject received the 16α -labeled steroid and on the third day, the 2- labeled material. After intravenous administration of each isotope, by a one-minute bolus infusion through an intravenous line in the antecubital fossa, serial blood specimens were obtained over a 48-hour period. The blood samples were frozen and lyophilized; 3 or 4 ml aliquots of the lyophilized water were counted in duplicate using a Packard 2650 counter. The $^3\text{H}_2\text{O}$ activity (SA) expressed as disintegrations per minute (dpm)/ml was calculated. A control blood sample was obtained immediately prior to the administration of each tracer.

Total body water in each subject was determined using a stable isotope labeled technique by the Rockefeller University NIH-DRR Mass Spectrometric Research Resource measuring the enrichment of body water with deuterium labeled water. In this technique, 75 ml of deuterium oxide (“heavy water”) was administered orally as an acute bolus to each subject and blood specimens were collected 3 hours later. Patients were maintained supine for 1 to 2 hours after the heavy water was given, to avoid the dizziness that has sometimes been reported to be associated with deuterium administration. No adverse reactions were experienced. The body water volume was then calculated from these data.

The body water volumes were used to calculate the total extent of the estrogen biotransformation reactions by multiplying the $^3\text{H}_2\text{O}$ SA and the body water volume and dividing by the dose administered.

$$\% \text{ reaction} = \frac{{}^3\text{H}_2\text{O SA (Bf Bo} \times \text{Bw} \times 100}{\text{Bf Bo} \times \text{Bw} \times 100}$$

Dose where SA=specific activity; Bf=48 hr dpm value; Bo--the zero time dpm value; and Bw = total body water volume. Dose = the total dose of labeled steroid administered. Statistical analyses of the data were carried out using the NIH supported Prophet System.

Results

Biotransformation at the 16 α and 2 positions respectively yield 16 α -hydroxyestrone, an estrogenic metabolite, and 2-hydroxyestrone, a weakly estrogenic or antiestrogenic metabolite. The extent of hydroxylation at the C-16 α and C-2 positions in otherwise healthy subjects is summarized in Table 2. The data from studies in different subsets of subjects with defined hepatic disease are summarized in Table 3. These results were compared with those obtained in normal male and female control subjects by statistical analyses.

These results show marked significant differences in the estrogen metabolism patterns in study subjects with different defined types of liver disease. Study subjects with acute and subacute viral hepatitis (Group 1), subjects who were clinically "silent carriers" of hepatitis B virus (Group 2) and also subjects who had biopsy-documented chronic active hepatitis of the "non-A, non-B type," (probably hepatitis C) (Group 3) showed a pattern of biological hydroxylation of estrogen not significantly different from normal subjects, with the extent of hydroxylation at both the 16 α and the 2 positions being similar to what was observed in the normal subjects.

In female patients with the clinical laboratory, serological, as well as biopsy, findings compatible with autoimmune hepatitis (Group 4), a significant reduction in reaction at the 2 position ($p < 0.01$), which would yield weakly estrogenic or antiestrogen "catechol" estrogens, was observed. There were no significant changes in the extent of the reaction at 16 α in these patients. However, reduction of the weakly estrogenic or antiestrogenic metabolites could lead to a relative increase in estrogenicity in this group. In the 6 subjects with alcoholic cirrhosis (Group 5), a highly significant reduction in the oxidative reaction at the 2 position was seen ($p < 0.01$) which would result in decreased production of the 2-hydroxylated weakly estrogenic or antiestrogenic "catechol" metabolites. This was accompanied by a significant reciprocal increase in the reaction at the 16 α position, which would yield a significant increase in the amount of the highly estrogenic metabolite, 16 α -hydroxyestrone and its further metabolite estriol. Since all of these subjects with chronic alcoholic cirrhosis were free of recent alcohol use, the observed effect of reduced 2-hydroxylation, as well as increased reaction at the 16 α position, cannot be considered to be a direct alcohol effect, but rather must be secondary to the presence of severe alcoholic cirrhotic liver disease. The decline in 2-hydroxylation may not simply reflect an overall decrease in hepatic function, despite the severity of liver disease in these subjects, since 16 α -hydroxylation which also is in part a hepatic enzymatic biotransformation pathway, was significantly increased in these subjects. Alternatively, 16 α -hydroxylation may be occurring at some peripheral site, such as the gastrointestinal mucosa and wall, the synovium or even the kidney (2,12,24).

Discussion

Feminization is a common finding in patients with severe chronic liver disease, especially alcoholic cirrhosis, in which setting it is accompanied by evidence of hypogonadism. The evidence of feminization includes appearance of spider angiomas, palmar erythema, female

hair distribution, female fat distribution, and gynecomastia (6–9). In the setting of alcoholic cirrhosis, impotence and testicular atrophy also occur (11). Concomitant hypogonadism in cirrhotic patients was initially related directly to the feminization, but recent studies have suggested that many different direct and indirect alcohol effects, as well as direct and indirect effects of severe cirrhotic liver disease per se, may contribute to each of these two findings (4–12,14,15). It has now been documented that there are direct deleterious effects of excessive alcohol use on the Leydig cells of the testis, resulting in decreased production of testosterone. Also, there are direct effects of alcohol on the hypothalamic-pituitary part of the hypothalamic-pituitary-gonadal axis, resulting in altered feedback control response mechanisms, with decreased responsiveness to gonadotrophin-releasing hormone and resultant inadequate increase in the levels of luteinizing hormone (LH), which regulates production of testosterone, and follicle-stimulating hormone (FSH), which helps regulate spermatogenesis (7,9,11,14,15). It has also been documented that these phenomena, which contribute to hypogonadism, may occur during alcohol abuse in the absence of any significant alcoholic liver disease (7,14).

Many studies have been performed in an attempt to define the mechanisms underlying the feminization observed in the setting of chronic liver disease. Early studies were focused on static measurements of urinary or plasma levels of estradiol or one or more of its metabolites, primarily estrone or estriol, with the hypothesis that impaired estrogen clearance or enhanced estrogen production, coupled with decreased testosterone production, might be the cause of the feminization observed (2,4,6,7). From these early studies, it was recognized that in hepatic cirrhosis there was an estrogen-androgen imbalance, with significantly increased amounts of active estrogens coupled with a reduction of total androgens. However, in other studies, diverse results were found, with most studies reporting significant increases in plasma levels of the primary estradiol metabolite, estrone, in the setting of cirrhosis, either of an alcoholic or post viral type, along with reduced levels of testosterone in those settings, with many reports finding normal or only slightly elevated levels of estradiol itself present in patients with cirrhosis due to alcohol or post-viral etiologies. Further studies on alcohol effects, with or without the presence of chronic liver disease which might contribute to feminization, included the findings of increased adrenal production of the weak androgen precursor dehydroepiandrosterone, increased levels and increased production of androst-4-ene-3, 17-dione. Increased peripheral conversion of these two weak androgens to estradiol and estrone occurred because of increased levels of the tissue aromatase enzyme, coupled with the well-established increased NADPH and reduced NADH in the setting of chronic alcohol abuse (2,4,6).

In the rat model, it has been shown that subacute (4 days) administration of alcohol causes a reduction in 2-hydroxylation in the liver, resulting in decreased formation of 2-hydroxylated estrogen metabolites (10). Restoration of enzyme activity was shown to occur within two weeks after cessation of alcohol administration (10). In other studies it was shown that portal hypertension, with resultant portosystemic shunting, could result in increased plasma levels of estrogens, even in the absence of liver disease (5,14). It was hypothesized that such shunting could result in decreased enterohepatic circulation of androgens and estrogens, thereby reducing not only biotransformation by the liver, but also by the intestinal mucosa, which has been shown to be an important site for the metabolism of these hormones (3,16,24). Other early as well as more recent studies, focused on the possible relationship between increased levels of estrogen or decreased levels of the testosterone. That is, the estrogen/testosterone ratio and the development of hepatocellular carcinoma in patients with cirrhosis, either in alcoholic or post-viral type (13,17). These authors found that in the setting of severe cirrhotic liver disease there is a consistent significant reduction in testosterone levels, a slight increase in estradiol levels, and a highly significant increase in

estrone levels in plasma, with a resultant highly significant decrease in the testosterone/estrogen ratio.

At the time of the very earliest studies of estrogen metabolism by the liver, it was thought that estradiol was metabolized primarily to estriol, which was then excreted in the urine; the possibility of biologically active intermediate products of oxidation was not considered. By 1960, it was shown that, in humans, estradiol is metabolized to estrone, which in turn serves as a substrate for both hydroxylation at the C-2 position to form catechol estrogens, and at the C-16 α position to form 16 α -hydroxylated metabolites (25,26). Subsequent studies showed that these products were formed to a far greater extent than could be estimated by determining urinary estriol concentrations alone (27). Further research showed that biotransformation of estrone by 2-hydroxylation is competitive with 16 α -hydroxylation and vice versa (2,6,28). By static studies in which urinary estrogen metabolites were measured, it was shown that in the setting of increased thyroid hormone levels 2-hydroxylation is increased and 16 α -hydroxylation is decreased (29). In similar studies, it was shown that in the setting of hepatic cirrhosis, 2-hydroxylation is decreased and 16 α -hydroxylation is increased (2). However, these were all static studies and not dynamic studies of estrogen metabolism in humans.

The failure of 2-hydroxylation of estrone in the subjects with alcohol cirrhosis in this study with a concomitant increase in reaction at the 16 α -position, may suggest a very specific inhibition of 2-hydroxylation and not simply a generalized failure of liver function. Similarly, the reduction in 2-hydroxylation, but with no change in either direction in 16 α -hydroxylation in patients with chronic active hepatitis of the autoimmune type found in this study also suggests a specific defect in this pathway.

The marked decrease in 2-hydroxylation, coupled with significantly increased 16 α -hydroxylation, found in alcoholic cirrhotic subjects would be expected to result in both a relative and an absolute increased amounts of the highly estrogenic 16 α -hydroxylated metabolites coupled, with a significant decrease in the weakly anti-estrogenic 2-hydroxylated catechol estrogens. These findings therefore offer an additional basis for the feminization observed in cirrhosis. These findings in the setting of alcoholic cirrhosis may be due in part to peripheral biotransformation of estradiol by hydroxylation at the 16 α position, possibly by the gastrointestinal mucosa or wall (3,16,24). These findings are also in accord with recent findings by Telang et al that 16 α -hydroxylation is present in the terminal duct lobular unit of the breast when grown in organ culture (31). In this study, all of the cirrhotic patients had a past history of alcoholism. However, since chronic hepatic damage with the severity of cirrhosis may occur as a consequence of a viral hepatitis of B, delta, and Non-A Non-b types alone or in combination with alcohol, further dynamic studies will need to be performed to document whether or not significant alterations in estrogen metabolism occur in those settings.

Other studies (30) have suggested that the potentially profound biological importance of these findings is that the catechol estrogens were shown to be only weakly estrogenic and even antiestrogenic in some settings, whereas the 16 α -hydroxylated metabolites have been shown to be highly estrogenic (30,31). Thus, as had been suggested by Van Thiel and colleagues (9), in the setting of hepatic cirrhosis, it is possible that part of the feminization may be due to a relative decrease in the 2-hydroxylated weakly estrogenic metabolites; according to our own hypothesis, based in part on our earlier studies of estrogen metabolism, this may be accompanied by a significant increase in the 16 α -hydroxylation pathway. The findings of our current study show that these hypotheses are both correct. In the setting of severe liver disease of an alcoholic type, i.e. alcoholic cirrhosis, but studied at a point in time (3 months) after cessation of alcohol use, a highly significant increase in the

estrogenic 16 α -oxidation pathway was observed, coupled with a very significant reduction in the weakly estrogenic or antiestrogenic 2-hydroxylated pathway, when the more reliable, dynamic radiometric assays for the determination of oxidative metabolism of estrogen were used in human subjects.

Since the development of these dynamic techniques for studies on the kinetics of estradiol biotransformation, it has been shown that there is a significant decrease in catechol estrogen formation in the setting of obesity, and also in subjects receiving treatment with the histamine receptor type 2 blocking agent, cimetidine, which is used in the treatment of peptic ulcer (20,23). Similarly, it has been shown that there is a highly significant increase in 16 α -hydroxylation of estradiol in systemic lupus erythematosus (20). The studies of patients with systemic lupus erythematosus were further confirmed by direct measurements of 16 α -hydroxyestrone, the major metabolite, using a radioimmunoassay technique (28). It has also been shown that significant alteration of dietary protein and carbohydrate may alter these principal biological pathways of estradiol metabolism in humans (21). Also, 2-hydroxylation was shown to be stimulated by smoking yielding a relative, anti-estrogen effect (22).

The failure of 2-hydroxylation of estrone in the subjects with alcohol cirrhosis in this study with a concomitant increase in reaction at the 16 α -position, may suggest a very specific inhibition of 2-hydroxylation and not simply a generalized failure of liver function. Similarly, the reduction in 2-hydroxylation, but with no change in either direction in 16 α -hydroxylation in patients with chronic active hepatitis of the Lupoid type found in this study also suggests a specific defect in this pathway.

The new findings of this study, which are based on dynamic studies of estradiol oxidative metabolism in male and female subjects with liver disease, and not simply on static measurements of plasma and urinary levels of deranged estrogen metabolites, confirm many earlier hypotheses, as well as much more recent hypotheses, concerning the possible role of altered estrogen metabolism in the feminization observed in the setting of chronic liver disease. The significant increased biotransformation to more estrogenic metabolites, which in turn would be expected to alter the responsiveness of LH to gonadotrophin releasing hormone, and possibly alter the release of gonadotrophin-releasing hormone itself, may add yet an additional explanation to the hypogonadism observed in the setting of alcoholic cirrhosis.

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Radiometric Measurement of Estrogen Metabolism

The extent of reaction is determined by the amount of tritium water formed.
Therefore, the extent of reaction is independent of the fate of the metabolites

Figure 1. Radiometric measurement of estrogen metabolism. The extent of reaction is determined by the amount of tritium water formed. Therefore, the extent of reaction is independent of the fate of the metabolites.

Table 1

Laboratory Findings in Study Subjects with Liver Disease

DIAGNOSIS	Liver Biopsy	AST	ALT	Bilirubin total	ALP ¹	Total Protein	Alb	ANA ²	AMA ³	HBsAG
Normal										
1. Acute Viral Hepatitis (³)										
F Va	No	798	201	3.8	228	8.2	3.6	Neg	ND	+
M Ge	No	2110	2600	18.9	230	7.5	4.3	ND	ND	+
M Vi	Yes	275	890	12.6	578	6.3	4.00	Neg	ND	+
2. Silent B Carrier(²)										
M Lu	Yes	66	121	0.8	103	7.1	4.2	ND	ND	+
M BU	No	36	ND	0.6	65	8.7	4.3	ND	ND	+
3. Non-Anon-Behronic AH(³)										
M Kr	Yes	762	8892	1.4	91	7.2	3.3	Neg	ND	
F Ma	Yes	67	ND	10.8	120	6.9	3.9	Pos	Neg	
F SI	Yes	41	128	0.7	256	6.9	4.1	Neg	Nd	
4. "Lupoid" (⁵) hepatitis CAH										
F Fe	Yes	57	ND	1.6	1.3	8.3	4.3	Pos	Neg	
F FI	Yes	152	234	0.7	70	8.1	3.7	ND?	ND?	
F JA	Yes	40	39	0.9	71	7.1	4.6	Pos	Neg	
F Re	Yes	ND?	ND?	ND?	ND?	ND?	ND?	ND?	ND?	
F Sm	Yes	19	20	0.4	76	ND?	ND?	Neg	Pos	
5. Chronic Alcoholic Cirrhosis (⁶)										
F Ga	Yes	97	ND	1.4	154	9.7	3.5	1+	Pos	
M Gr	Yes	37	ND	0.5	134	7.3	3.3	ND	ND	
M Ma	Yes	90	ND	3.1	305	7.5	2.7	ND	ND	
M Ri	Yes	52	ND	0.6	220	7.5	2.7	ND	ND	
M To	Yes	34	ND	0.9	109	6.9	3.7	ND	ND	
F Yo	No	0.3	ND	4.0	120	6.1	3.4	NdND		

¹ ALP = Alkaline Phosphatase

² ANA = Anti-Nuclear Antibody

³ AMA = Antimitochondrial antibody

⁴ HB_sAg hepatitis B surface antibody

⁵ ND = not done

⁶ ND? + Tet done but results either not reliable or not reported

Nona-Nonb chronicAH probably = hepatitis C but the test was not done.

TABLE 2

ESTROGEN HYDROXYLATION IN HEALTHY CONTROL STUDY SUBJECTS AND PATIENTS WITH VARIOUS LIVER DISORDERS

	20HE1	16OHE1
Females (pre and post menopausal)	33.9±2.5* (N=21)	10.3±0.6 (N=27)
Males	22.3+1.3 (N=28)	8.9±0.5 (N=30)
Combined Control Group	27.3+1.5 (N=49)	9.5+0.4 (N=57)

Group	Diagnosis	Sex	2-OH	16-OH
1	Acute and Subacute	F	31.8	13.4
	Viral Hepatitis B	M	19.4	
		M	37.1	9.7
2	“Silent” Hepatitis	M	19.8	13.7
	B Carrier	M	21.5	9.7
3	Non A-Non B	M	26.2	11.6
	Chronic Active	F	40.7	7.0
	Hepatitis	F	48.9	11.6
Mean (± SEM): Groups 1,2,3			30.7(±3.8)	11.0(±0.9)
Significance			NS	NS
4	“Lupoid” hepatitis	F	14.3	15.0
		F	20.4	9.0
		F	19.5	14.9
		F	9.9	13.2
		F	17.3	11.8
	Mean (±SEM)		16.3(±1.9)	12.8(±1.1)
Significance			P<0.01	NS
5	Chronic Alcoholic	F	3.6	16.3
	Cirrhosis	M	4.4	12.9
		M	2.0	10.7
		M	1.9	
		M	4.0	20.0
		F	1.3	17.7
Mean (±SEM)			2.9(±0.5)	15.5(±1.7)
Significance			p<0.01	p<0.01

* percent of labeled estradiol metabolized by each specific hydroxylation pathway; m ± SEM (number of subjects studied).

Percent of radiolabelled estradiol metabolized by specific hydroxylation pattern ± SEM.