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THE EFFECT OF ETHANOL ON
CORTISOL METABOLISM IN MAN

A thesis presented in fulfilment of the
requirements for the degree of
Master of Science in Biochemistry
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ABSTRACT

Methods were developed for the estimation of human plasma cortisol by radioimmunoassay and urinary 6 β -hydroxycortisol (6 β OHF) by colorimetry after separation by thin layer chromatography (TLC). In addition profiles of urinary neutral steroids were obtained by gas chromatographic separation of methoxime-trimethylsilyl derivatives from urine extracts on a glass capillary column. This approach was found to be more sensitive and reproducible than profile studies based on TLC separation and colorimetric estimation.

Pilot studies of the plasma cortisol levels of normal subjects showed a consistent rise in cortisol during alcohol loading under the conditions of the observations, but in hospital patients admitted with acute alcohol intoxication, variability in the experimental conditions masked any consistent changes. Large variations in method reproducibility as well as subject differences affected results from the measurement of 6 β OHF and chloroform extractable 17-hydroxycorticosteroids in one normal and four alcoholic subjects, rendering apparent initial differences insignificant. The results suggest, but do not demonstrate, that alcohol ingestion may divert normal cortisol metabolism into a pathway leading to the production of 6 β OHF.

Urinary steroid profiles obtained from two normal subjects, one normal subject under conditions of alcohol load and one alcoholic subject suggest that any effects of alcohol on cortisol metabolism are subtle and would require study of a large number of cases to define them.

This work has served to delineate the faults and potential of various approaches to the study of cortisol metabolism and the possible effects of alcohol thereon. It would seem that their application in carefully designed and well controlled experiments to a larger number of subjects is necessary to obtain the information desired.

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ABBREVIATIONS

GENERAL

A.C.T.H.	adrenocorticotrophic hormone
BTZ	Blue Tetrazolium (chloride)
CBE	cholesterol n-butyl ether
TSIM	trimethylsilyl imidazole
TMS	trimethylsilyl
GC	gas chromatography
GLC	gas liquid chromatography
TLC	thin layer chromatography
PC	paper chromatography
RIA	radioimmunoassay
Kg	Kieselghur
Si gel	silica gel
17-KS	steroid with keto group at 17-carbon position
17-OHCS	steroid with hydroxyl group at 17-carbon position
EtOAc	ethyl acetate
EtOH	ethanol
MeOH	methanol
CH ₂ Cl ₂ , DCM	dichloromethane
C-19	steroid with no side chain at carbon 17
C-21	steroid with 2 carbon side chain at carbon 17

STEROIDS

<u>Abbreviation</u>	<u>Trivial name</u>	<u>Systematic name</u>
An	androsterone	5 α -androstan-3 α -ol-17-one
Et	etiocholanolone	5 β -androstan-3 α -ol-17-one
11-HAn	11-hydroxyandrosterone	5 α -androstan-3 α ,11 β -diol-17-one
11-HEt	11-hydroxyetio- cholanolone	5 β -androstan-3 α ,11 β -diol-17-one
11-KAn	11-ketoandrosterone	5 α -androstan-3 α -ol-11,17-dione
11-KEt	11-ketoetiocholanolone	5 β -androstan-3 α -ol-11,17-dione
DHEA	dehydroepiandrosterone	5-androsten-3 α ol-17-one
Pd	pregnanediol	5 β -pregnan-3 α ,20 α -diol
Pt	pregnanetriol	5 β -pregnan-3 α ,17 α ,20 α -triol
Atr	androstenetriol	5-androsten-3 β ,16 α ,17 β -triol

E	cortisone	4-pregnen-17 α ,21-diol-3,11,20-trione
F	cortisol	4-pregnen-11 β ,17 α ,21-triol-3,20-dione
THE	tetrahydrocortisone	5 β -pregnan-3 α ,17 α ,21-triol-11,20-dione
THF	tetrahydrocortisol	5 β -pregnan-3 α ,11 β ,17 α ,21-tetrol-20-one
a-THF	allo-tetrahydrocortisol	5 α -pregnan-3 α ,11 β ,17 α ,21-tetrol-20-one
α Co	α cortolone	5 β -pregnan-3 α ,17 α ,20 α ,21-tetrol-11-one
β Co	β cortolone	5 β -pregnan-3 α ,17 α ,20 β ,21-tetrol-11-one
α Cor	α cortol	5 β -pregnan-3 α ,11 β ,17 α ,20 α ,21-pentol
β Cor	β cortol	5 β -pregnan-3 α ,11 β ,17 α ,20 β ,21-pentol
6 β OHE	6 β hydroxycortisone	5-pregnen-6 β ,17 α ,21-triol-3,11,20- trione
6 β OHF	6 β hydroxycortisol	5-pregnen-6 β ,11 β ,17 α ,21-tetrol-3,20- dione

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CHAPTER I

GENERAL INTRODUCTION

The Effects of Alcohol on Human Endocrine Function

From reviews of this topic, such as that of Wright (1978), it may be concluded that the effects of ethyl alcohol on hormone secretion and metabolism are not large and dramatic, with the exception of its direct inhibition of the neurosecretion of the neurohypophyseal hormones vasopressin and oxytocin. However, as shown from alcohol consumption figures, the average adult in any western society has ethanol present in his bloodstream for several hours per day throughout life, and the tissues of many are never ethanol-free. Under these conditions, even minor disturbances of endocrine balance may become clinically significant and worthy of investigation.

The concentration of hormone to which a receptor tissue responds may be influenced by ethanol if this either (a) affects the rate of secretion of the hormone from the source tissue, or (b) modulates its rate of catabolism or metabolic activation, particularly if this occurs in the liver where ethanol is actively oxidized to acetaldehyde and acetate. Examples of both types of interaction have been reported.

Assessment of the Literature

In spite of the considerable volume of literature on the endocrine consequences of alcohol ingestion, its interpretation is complicated by a number of problems. Comparison of the changes involving acute administration of alcohol with those due to prolonged intake, such as occur in chronic alcoholism, and comparison of the response of habitual drinkers with the response of alcohol-naive subjects, has led to confusion and apparently conflicting results. In addition there has frequently been a failure to distinguish the endocrinological and metabolic effects of alcohol per se from those secondary to tissue (particularly liver) damage and a tendency to regard chronic alcoholics as a homogeneous group regardless of differences in drinking patterns, the type and quantity of liquor consumed, the history, nutritional status and the time interval between drinking and endocrine or

metabolic studies, all of which may profoundly affect the results obtained. Finally there is some difficulty in assessing data obtained before the introduction of modern hormone assays such as specific radioimmunoassays and of correlating results obtained from animal and human studies.

Hypothalamic-Pituitary-Gonadal Function

Hepatic cirrhosis in men is commonly associated with both hypogonadism and feminization. The similarities between the endocrine features of alcoholic and non-alcoholic cirrhosis initially suggested that it was the liver disease itself which was responsible for these changes. Recent evidence, however, suggests a possible direct effect of alcohol on testicular function. The changes described so far indicate that gonadal dysfunction may occur in the absence of overt liver disease but that in alcoholic cirrhosis, the cumulative effects of alcohol and hepatic dysfunction may produce more marked endocrine features. The subject is covered in some detail in the reviews by Adlercreutz (1974), van Thiel and Lester (1976) and Green (1977).

Secretion of Catecholamines

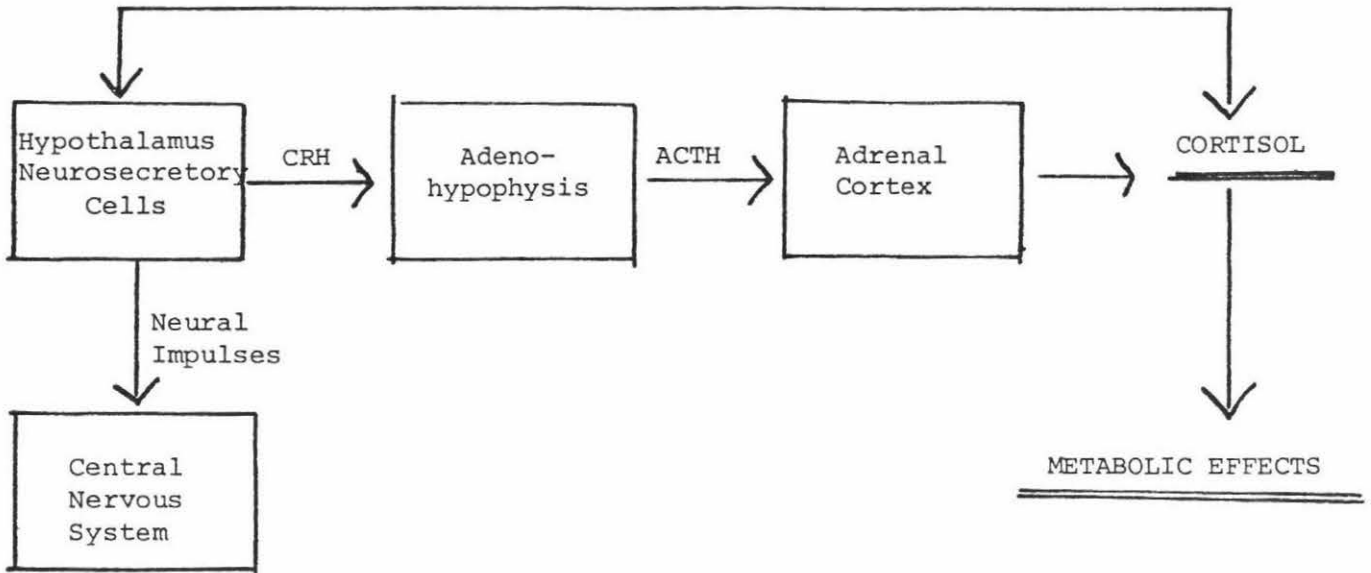
There is evidence from both human and animal studies that alcohol stimulates adrenal medullary secretion. Moderate doses of alcohol have been shown to produce a rise in both the plasma and urine catecholamines of normal human subjects (Perman, 1958; Anton, 1965), while similar effects have been observed in subjects with prolonged histories of drinking (Ogata et al, 1971).

Hypothalamic-Pituitary-Adrenocortical Axis

The effects of alcohol on endocrine function have been studied most extensively in relation to the hypothalamic-pituitary-adrenal (H.P.A.) axis and work in this field has been reviewed by Schenker (1970), Marks and Chakraborty (1973) and Wright (1978). The system and its regulation is shown schematically in Fig. 1i.

Figure 1i

Regulation of the Hypothalamic-Pituitary-Adrenal Cortex System



Short loops may exist between hypothalamus-pituitary and pituitary-adrenal, imposing further regulation.

Reproduced from Marks and Chakraborty (1973).

Cortisol is the major adrenocortical steroid hormone found in human blood. The circulating levels of cortisol have been shown to rise rapidly in response to trauma e.g. injury, surgery, burns etc. (as reviewed by Alberti and Johnston, 1977). Cortisol is the major anti-anabolic hormone: its ability to inhibit protein synthesis is thought to be responsible for its unique anti-allergic and anti-inflammatory effects. The secretion of cortisol is under the direct control of adrenocorticotrophic hormone (A.C.T.H.) produced by the adrenehypophysis (anterior pituitary) in response to the neuroendocrine releasing factor C.R.H. (corticotrophin releasing hormone). The release of C.R.H. is, in turn, determined by the action of external stimuli on the central nervous system as well as a circadian "clock".

A Review of the Literature on the Effects of Alcohol on Cortisol Release and Metabolism

Although H.P.A. function appears to be definitely disturbed in chronic alcoholics (Stokes, 1971) the literature reports are often contradictory. This appears to be due, in part, to the absence of suitable techniques for measuring the hormones involved, as well as the multiplicity of possible physiological, psychological and sociological contributions.

In man the effects appear to be dose related: while moderate to large doses may activate adrenocortical activity through higher regulatory centres (rather than by direct action on the adrenal or pituitary), lower doses are less predictable and it has been postulated that they may even decrease the activity of a previously aroused H.P.A. system via a sedative effect on the central nervous system.

Kissin et al. (1959) suggested that some of the observed abnormalities in the adrenocortical function of alcoholic subjects may be related to impaired liver function. A further investigation (Kissin et al., 1960) demonstrated increased urinary 17-OHCS and decreased plasma levels, accompanied by a marked diuresis, within two hours of a single dose of ethanol (1 g/Kg body weight) to alcoholic subjects. The similar effects of a water load seemed to indicate that the adrenocortical depletion may have been due to increased renal clearance, but a simultaneous water and

ethanol load produced a rise in plasma 17-OHCS with no appreciable change in urinary levels, suggesting an active stimulatory effect of ethanol on the adrenal cortex.

Perman (1961) however, failed to show a significant change in urinary 17-OHCS two to three hours after a 1 g/Kg dose of ethanol to non-alcoholic subjects; there were no corresponding plasma steroid measurements.

Margraf et al. (1967) found no significant difference in cortisol secretion rate or total excretion of 17-OHCS in alcoholic subjects as compared with non-alcoholic controls, although the distribution of the individual component steroids appeared to differ significantly from normal. In addition, 24 hour 17-ketosteroid excretion, response to A.C.T.H. and rate of metabolism of exogenous cortisol appeared to be lowered in alcoholics, while plasma corticosterone and its urinary metabolites were increased above normal levels, suggesting that alcohol affected steroid metabolism rather than adrenocortical function.

In reviewing the literature Schenker (1970) suggests that chronic alcoholics show a plasma 17-OHCS level significantly higher than that of partially rehabilitated alcoholics, which in turn, is higher than that of non-alcoholics. A marked rise in an alcoholic's plasma 17-OHCS is often associated with gastro-intestinal disturbance or withdrawal. After 12 hours without alcohol acutely withdrawn, chronic alcoholics showed a 9 am plasma cortisol significantly higher than normal, which fell following the ingestion of small amounts of alcohol (Merry and Marks, 1972). This compares with a distinct rise in plasma cortisol following infusion (Jenkins and Connolly, 1968) or ingestion (Merry and Marks, 1969; Bellet et al., 1970) of ethanol to/by normal subjects. These findings suggest that withdrawal represents a state of considerable stress to the alcoholic the symptoms of which may be relieved by alcohol. Alcohol ingestion by non-alcoholics, however, raises plasma cortisol levels probably by increasing pituitary-adrenocortical activity, since no such effects were noted in non-alcoholic patients with clinical adrenal insufficiency (Bellet et al, 1970).

The Aim of this Project

The goal of the present research was to elucidate some of the effects of ethanol consumption on adrenal corticosteroid release and metabolism in an endeavour to clear up some of the apparent inconsistencies in the literature. Initially, attempts were made to cover effects on both the plasma level of cortisol and its conversion to metabolites and to study both normal and alcoholic subjects. Both approaches required establishment of modern methods of analysis, which occupied most of the time available for this project.