STUDIES ON THE RELATIONSHIP BETWEEN SOME TRACE ELEMENTS AND STEROID HORMONES IN PROSTATIC DISORDERS AMONGST NIGERIANS

A THESIS SUBMITTED TO THE DEPARTMENT OF CLINICAL PATHOLOGY, UNIVERSITY OF LAGOS IN PART FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (ENDOCRINOLOGY)

BY

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CERTIFICATION

This is to certify that the Thesis titled

"Studies on the relationship between some trace-elements and steroid hormones in prostatic disorders amongst Nigerians"

submitted to

The School of Postgraduate Studies of

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is a record of Original Research carried out by

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Date: OCTOBER, 1986

BETWEEN THE IDEA

AND THE REALITY

BETWEEN THE MOTION

AND THE ACT

FALLS THE SHADOW

T. S. ELLIOT

DECLARATION

The works contained in this study were undertaken entirely by me. No part of this study has been presented, either in part or as a whole, to any other Institution or Organisation for the purposes of obtaining any degree or qualification.

J.O. OGUNLEWE
CANDIDATE

PROF. A.C. ONITIRI
SUPERVISOR

D E D I C A T I O N

This THESIS is dedicated to :

My Parents, Amos Olugbosi and Mabel AJOKE

My Brothers, Bola, Akin, Seye and Leke

My Sister - Adenike

My Teachers - Femi, Dotun Oyewole, Jim Pescod, Prof. Lyne Reid and Prof. Stitch

My Wife - Cecille Olabisi

and Children - Babatunde, Aderinola, Adeyanju, Adepeju and Adebowale.

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ABBREVIATIONS

The abbreviations used in this thesis are in accordance with the Policy and Instructions to Authors, Biochemical Journal, 1976, Volume 153 pages 1 - 21.

Other abbreviations are as follows:

A-DIONE	-	Androstenedione
DIOLS	-	Androstanediol (3α(β))
DHT	-	Dihydrostestosterone
Cpm	-	Counts per minute
I : d	-	Internal diameter
mg	-	Milligram (10^{-3} gram)
ug	-	microgram (10^{-6} gram)
ng	-	nanogram (10^{-9} gram)
pg	-	picogram (10^{-12} gram)
Mc	-	Millicurie (10^{-3} curie)
uc	-	microcurie (10^{-6} curie)
mmol	-	Millimol - (10^{-3} Mol)
umol	-	micromol (10^{-6} Mol)
nmol	-	nanomol - (10^{-9} mol)
pmol	-	picomol - (10^{-12} mol)
PPO	-	2, 5 - diphenyloxazole
POPOP	-	1,4, bis - 2 - (4-methyl-5-phenyloxazolyl)-benzene
Rf	-	ratio to solvent front
Sp act	-	Specific activity
tlc	-	thin-layer chromatography
V/v	-	Volume per volume
W/v	-	Weight per volume

W/w	-	Weight per weight
Vol	-	Volume
^{3}H	-	Tritium
nm	-	nanometre
RIA	-	Radioimmunoassay
NADPH	-	Nicotinamide Adenine Dinucleotide Phosphate (reduced form)
DCM	-	Dichloromethane

STEROID NOMENCLATURE

The systematic names of the steroids whose trivial names are used in this thesis are in accordance with the IUPAC-IUB 1969 Revised Tentative Rules of Steroid Nomenclature.

TRIVIAL NAMES	ABBREVIATION	SYSTEMATIC NAMES
ANDROSTENEDIONE	A-DIONE	4-androstane-3, 17-Dione
5-Alpha-Androstanedione	5 α-DIONE	5 α-androstane-3-17,dione
5-alpha-Androstanediol (3-alpha)	3 α DIOL	5 α androstane, 3α, 17β diol
5-alpha-Androstanediol (3-Beta)	3 β-Diol	5 α androstane, 3β, 17β diol.
5-alpha-dihydro-testosterone	DHT	17β, hydroxy 5α androstane-3-one
Testosterone	T	17β hydroxy-4-androstane-3-one.

ENZYME NOMENCLATURE

The Nomenclature of the Enzymes measured in this Thesis are in accordance with the recommendations of the Commission on Enzymes of the International Union of Biochemistry, 1978.

TRIVIAL NAME	SYSTEMATIC NAME	EC NOMENCLATURE
5α reductase	Δ^4 3 Ketosteroid-5α reductase	EC 1 . 3 . 1 . 4
3α OHSD	3α Hydroxysteroid oxido-reductase	EC 1 . 1 . 1 . 50
17β OHSD	17β -Hydroxysteroid oxido-reductase	EC 1 . 1 . 1 . 63

ABSTRACT

Benign hyperplasia and Carcinoma of prostate are prostatic disorders commonly found in aging males. These disorders are reported to be less common amongst Nigerians, though there has been no concrete pathophysiological evidence to support this observation.

The exact aetiology of these disorders has not been resolved, but some factors have been identified as being contributory to their development. Amongst these factors are androgenic hormonal control and the influence of some trace-elements on the metabolic activities of the gland.

We investigated the interrelationship between these factors in Nigerians with a view to elucidating any physiological differences that may exist between Nigerians and other peoples.

Our results showed that the extracellular hormonal millieu of the prostate gland in Nigerians is not significantly different from those of Caucasians in Europe and North America. Plasma testosterone levels in healthy Nigerians, aged between 50 and 90 years (15.5±0.4nmol/l SE) is comparable to the levels reported for caucasians of a similar age group in Germany and England. This level is also significantly higher than the levels in age-matched cancer patients (10.9±0.7 nmol/l) ($P < 0.001$), but not significantly different from the levels in age-matched BPH patients (14.9±0.4 nmol/l SE; $P > 0.10$). Levels of other androgens were not different between the three groups. Plasma levels of zinc were higher in BPH subjects and lower in cancer patients compared with healthy controls, while

plasma cadmium was higher in cancer patients.

Intraprostatic DHT was elevated in hyperplastic tissues (4.9±0.2 ng/g) relative to normal tissues (1.7±0.18 ng/g) and malignant tissues (1.7±0.2 ng/g). In contrast, testosterone and A-Dione were found in greater concentration in malignant tissues (7.9±0.6 ng/g and 4.6±0.3 ng/g respectively) than in BPH (0.5±0.3 ng/g; 0.2±0.01 ng/g) and normal tissues (0.3±0.05 ng/g; 0.2±0.03 ng/g).

Zinc was significantly concentrated in hyperplastic tissues (17.9±0.6 umol/g or 1170±39.2 ug/g) and Normal tissues (12.1±0.8 umol/g; 791±55.5 ug/g) than in malignant tissues (2.9±0.4 umol/g; 189.6±26.1 ug/g). A greater proportion of zinc and DHT were located in the nuclear fraction of hyperplastic tissues. In contrast, the cytoplasmic concentration of zinc was inversely proportional to DHT concentration in these tissues. Cadmium accumulated more in malignant tissues (28.9±0.37 nmol/g) compared with normal (3.8±0.63 nmol/g) and hyperplastic tissues (14.6±1.1 nmol/g).

These results suggest an interrelationship between the concentration of zinc and accumulation of DHT, especially in the nucleus of hyperplastic tissues.

Results of *in vitro* experimental studies involving additions of varying concentrations of exogenous zinc and cadmium aptly supported the above findings. Additions of low concentration (10^{-5} to 10^{-12}M) of exogenous zinc enhanced the activities of 5α reductase and 3α hydroxysteroid dehydrogenase (Reductase) in the conversion of testosterone to DHT and DHT to 3α diol in hyperplastic tissues, whereas concentrations higher than 10^{-5}M inhibited the reaction. Similar results but to a lesser degree were

obtained with carcinomatous tissues. Experiments with cadmium also showed similar effects on the transformation of testosterone to DHT.

This study shows that the intracellular and extracellular hormonal environment of the prostate gland in Nigerians is not different from that of Europeans. However, the interrelationship between plasma and tissue concentrations of zinc and androgens, appears to affect the levels of androgens available to the prostate, which in turn could promote or prevent the processes culminating in prostatic disorders.

LEGENDS

FIGURES

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CHAPTER 4 (CONT'D.):

CHAPTER 1

INTRODUCTION

CHAPTER 1

1.1. GENERAL INTRODUCTION

The Adult Human Prostate - Anatomical Development and Pathogenesis of its disorders

The adult human prostate is a musculo-glandular organ which is in contact with the inferior surface of the bladder and is closely linked with other male sex accessory organs (Fig. 1.1 page 3). The gland reaches the adult size in the third decade of life when its average weight is 20g. However, by the age of 40 years, the muscles of the gland begin to change and the running fibres condense into a kind of septum which tends to divide the acini into "inner" and "outer" zones. With advancing age, the division becomes increasingly distinct and the behaviour of the zones, on either side of the septum tends to differ. Thus these zones have been closely linked with later development of prostate disorders.

The prostate in the aging male is susceptible to hyperplasia and malignancy, unlike other male sex accessory organs. The earliest and most constant manifestation of hyperplasia is the presence of lateral stromal nodules which arose within the tissues lying both proximal to the verumontanum and in close proximity to the cylindrical urinary Sphincter (M^{c}Neal, 1978). Other nodules formed in the area anterior or ventral to the urethra may develop progressively into massively enlarged glands (Fig. 1.2 page 6). In contrast, the majority of prostatic cancer is now known to arise in the peripheral or subcapsular tissue of the gland (Frank, 1973; Whitemore, 1973).

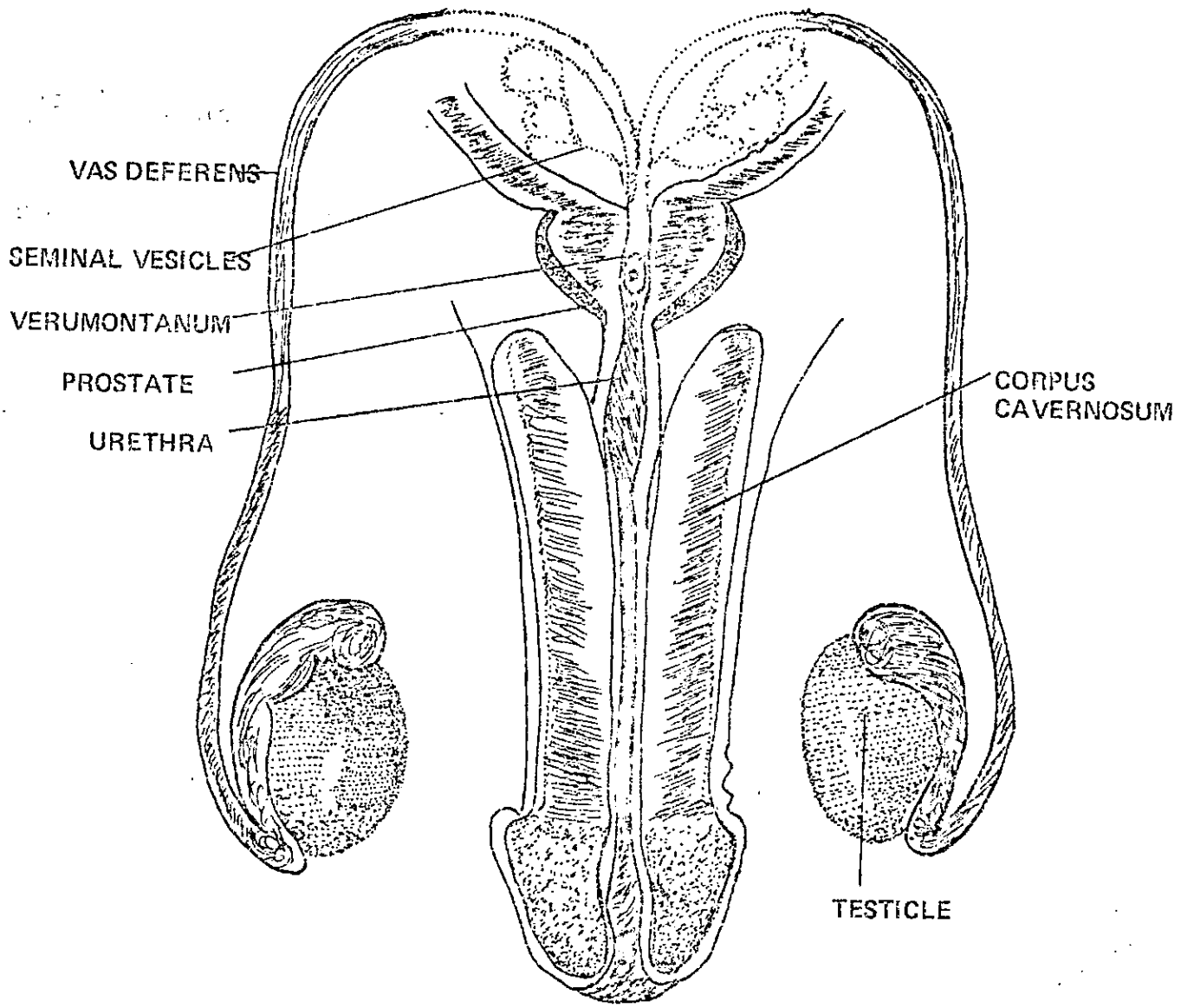


FIG. 1:1 - Normal prostate and other Male sex accessory organs.
(After D.R. Smith: general Urology 9th Ed. P. 2)

Incidence of Prostatic Disorders:

Since the recognition of benign prostatic hyperplasia (BPH) and Carcinoma of prostate (CaP) as pathological disorders of the prostate gland, it has been the general belief that these disorders are commonly found in aging males, especially after the seventh decade of life. However, studies have documented these disorders in age groups younger than 50 years in both blacks and caucasians (Amaku, 1976; Levine and Wilchinvsky, 1979).

It is remarkable that these disorders are occurring more commonly amongst Black Americans and they manifest themselves a decade earlier than in Caucasians (Levine and Wilchinvsky, 1979; Schuman and Mandel, 1980; Thind et al, 1982). However, a lower incidence rate than in Black Americans and Caucasians has been reported for Black Africans (Jackson, Ahluwalia, Herson et al, 1977; Ahluwalia, Jackson, Jones et al, 1981; Thind et al, 1982). Blacks in Bulawayo (Zimbabwe) have the highest observed incidence rate of CaP amongst Africans (32.0/100,000 population) but this figure is much lower than for blacks in Newark (NJ USA) which recorded 67.5/100,000 population (Thind et al, 1982). Reports on Nigerians showed an even lower incidence of 10.0/100,000 population (Thind et al, 1982).

However, studies in Nigerians have documented higher incidence of prostatic diseases in the population, contrary to above reports (Amaku, daRocha-Afodu, Elebute, 1971). These authors reported an incidence rate of 18.0/10,000 admissions for CaP, a situation similar to that reported for Europeans in England (Riches, 1962). The observation that incidence of prostatic disorders in Nigerians may be

higher than hitherto reported, appeared confirmed by Lawani, Nkposong, Aghaduno and Akute (1982). Their report based on admissions of tumours of Genito-Urinary tract into UCH, Ibadan between 1960 and 1979, recorded a frequency rate of 46.7% for CaP out of all registered cases of genito-urinary tract tumours.

Benign hyperplasia of prostate has also been observed to be on the increase among blacks (Schuman and Mandel, 1980); this disorder had earlier been recognised as a common cause of prostatic obstruction in Nigerians (Amaku et al, 1971).

Regardless of the relative incidence of these disorders amongst various population, it is of the greatest interest for us to understand the aetiological factors in their pathogenesis.

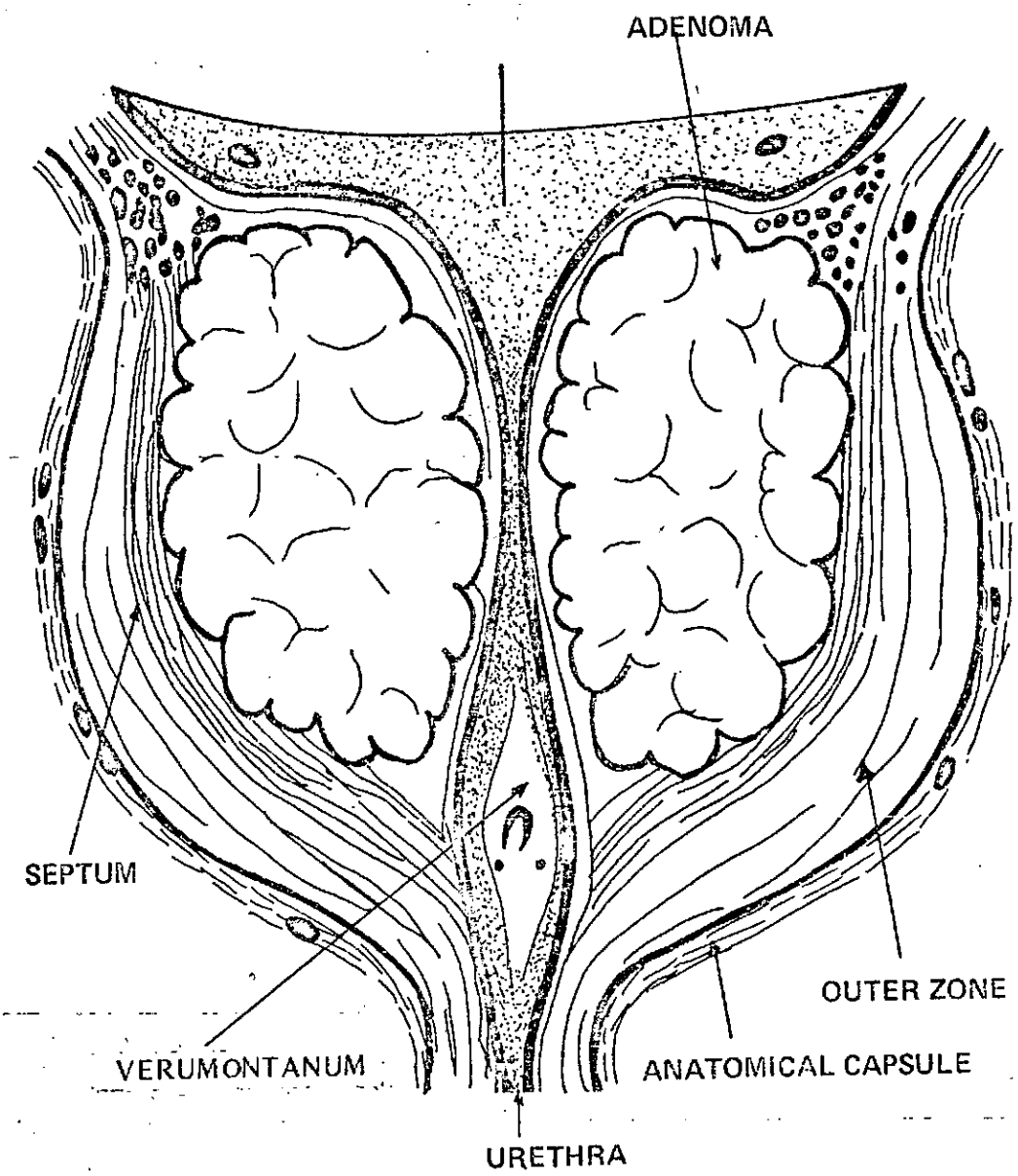


Fig. 1.2: Cross - sectional view of Adult Prostate showing the changes resulting from the development of adenoma in the inner zone of the gland.

(After J. Blandy: Urology Vol. II Pg. 862)

Studies of Aetiological Factors:

It has been established that the human prostate requires androgens for its normal development, hence there is a general belief that testicular hormones must play a critical role in the development of prostatic disorders. This belief was reinforced by the absence of prostatic disorders following castration. However, reports of determinations of circulating levels of testicular hormones have not established a causal association between plasma androgens and prostatic disorders.

Reports by Ofner (1970) that testosterone is metabolised in human prostate to dihydrotestosterone (DHT) gave indications that the changes resulting in disorders may be initiated within the gland itself. Therefore, determinations of the concentrations of testosterone and its metabolites were undertaken; and there is now a concensus that accumulation of DHT in the gland may be causal to the development of hyperplasia (Isaac, Charles, Brandler, Walsh, 1983). What is not known precisely is whether the DHT accumulation is the consequence of increased enzyme activity (5α reductase) or a defect in the further metabolism of this hormone.

Human prostate also contains a very high concentration of zinc (Leissner, Fjelkagard, Tissel, 1980; Tisell, Fjelkgard, Leissner, 1982). The concentration of zinc in this gland is androgen-dependent (Leissner et al, 1980), thus suggesting a link between prostatic zinc and androgens. Many studies have examined this relationship without conclusive results (Wallace and Grant, 1975; Habib, 1978).

Therefore, in the following pages, some of these

reports as well as studies of other biochemical factors are reviewed, and a framework for investigation of these factors in Nigerians is proposed. It is hoped that this study will elucidate the contributions of androgens to the development of prostatic disorders in Nigerians, and the roles of zinc and cadmium in promoting or preventing these disorders.

1.2. REVIEW OF LITERATURE

REVIEW OF LITERATURE

There have been inconclusive results regarding possible factors responsible for initiating the processes which culminate in the development of hyperplasia or malignancy of human prostate. However, some contributory indicators have been identified and explanations are being sought for their roles. These include amongst others:

(1) The endocrine control of prostate through the circulating androgens and possibly oestrogens, in view of the absence of BPH and CaP in men who are castrated or who develop hypopituitarism before the age of 40 years (Moore, 1944). Besides, there is the widely held belief that the disorders occur in men at the age when androgen secretion is on the decline (Vermuelen, Rubens and Verdoneck, 1972).

(2) The metabolic processes in the prostatic cell resulting in accumulation of intraprostatic androgen concentration, especially at the molecular level.

(3) The roles of some trace-elements (Zinc, Cadmium, etc.) in modulating the metabolic processes in the prostate with the resultant accumulation or depletion of hormones required for normal prostate development and physiological functions.

These factors have been investigated in the recent past in different parts of the world and it is now possible to review the accumulated evidence and to propose a hypothesis as the basis of this study. However, the involvement of other factors (race, genetics, diet, etc.) has been postulated but these will not be reviewed in any detail

since they are not relevant to the present study.

Androgens and Prostatic Disorders

That the growth and functional differentiation of the prostate is dependent on testicular function has been well known for many years. John Hunter in 1792 provided the earliest experimental evidence by showing that castration resulted in decrease in the size of the prostate glands. The report by Huggins and Stevens (1940) that castration of BPH patients produced histological atrophy of the prostate, not only confirmed earlier reports, but also provided the evidence for androgen involvement in prostatic diseases. Further evidence of the dependence of prostate on continued androgen stimulation was provided by Brandes (1974), who showed that androgen deprivation induces structural changes resulting in decrease or loss of metabolic and functional activities; a situation which was reversed by administration of testosterone. The major circulating plasma androgen is testosterone, which is secreted principally by the Leydig cells of the testis in response to stimulus from luteinising hormone (LH). The involvement of other gonadotrophins, especially prolactin, has been speculated and reports of many animal experiments have inferred a synergistic relationship between androgens and prolactin in promoting prostatic growth (Aragona, Bohnet and Friesen, 1977). However, this relationship has not been conclusively confirmed in humans.

Besides testosterone, there are other androgens in peripheral circulation. Some of these androgens are derived partly from testicular secretion and partly from the adrenals. Other sources of these androgens include

peripheral metabolism and interconvertibility of testosterone (Fig. 1.3, page 13). These androgens include Dihydrotestosterone (DHT) - a more "potent" androgen (than testosterone), androstenedione (A-Dione) androstanediol (Diols) and dehydroepiandrosterone (DHA). Studies by Ito and Horton (1971) and Ishiamaru, Pages, Horton (1977) have shown that between 70 and 80% of circulating DHT in healthy males is derived from peripheral metabolism of testosterone, while androstenedione is interconvertible with testosterone (Tremlay, Kowarsk, Park and Migeon, 1972; McDonald, Madden, Brenner et al, 1979).

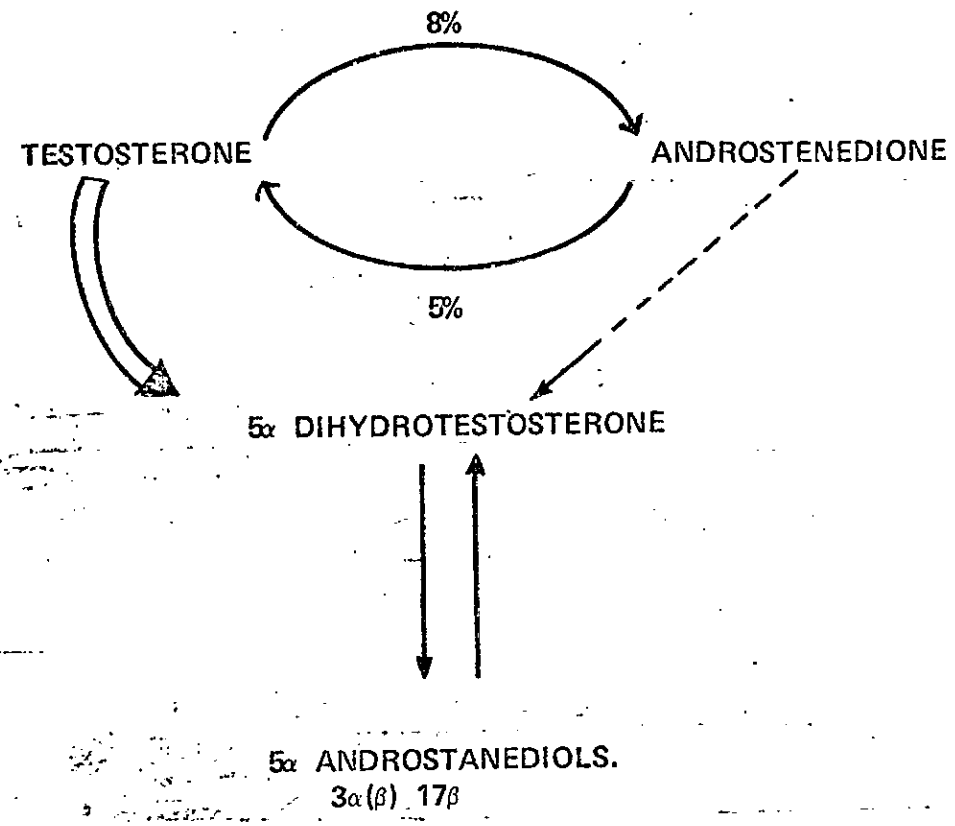


FIG 3: Interrelationship of plasma androgens involved in endocrine control of prostate growth.

Redrawn from Ito and Horton (1971); Ishiamaru et al, (1977); Tremlay et al, (1972) and McDonald et al, (1979).

It has also been shown that DHT is further metabolized to epimeric androstanediols. Furthermore, 17β-oestradiol is obtained through the activities of 17β hydroxysteroid dehydrogenase enzyme.

All these pieces of evidence suggest a very close relationship between testosterone, its metabolites and oestrogens. In fact, it has been suggested that the physiological consequences of testosterone are a combination of the effects of testosterone, its metabolites and oestrogen derivatives (Wilson, 1975). Therefore, a large number of studies have been undertaken to elucidate the endocrine milieu which may be conducive to the promotion of healthy prostatic function or protection against prostatic disease, by determining the plasma levels of these androgens in both healthy males and in those with prostatic diseases.

Therefore, the question may be asked. Do higher or lower levels of circulating testosterone or other androgens directly or indirectly promote or prevent the development of prostatic disorders? If these were so, determinations of the levels of plasma androgens should be a discriminant between healthy males and patients with prostatic diseases.

Reports of several studies, in this regard, have been inconclusive.

Plasma Androgens in BPH

Giusti, Gonnelli, Borrelli et al, (1975) have reported that testosterone level is decreased in spermatic vein with advancing age, thus advancing the expectation of low plasma testosterone levels in elderly BPH. Hence earlier studies from the U.S.A. showed significantly lower plasma testosterone levels in BPH compared with age-matched healthy men

(Farnsworth, 1971), and healthy young men (Horton, Hsieh, Barberia et al, 1975; Ishiamaru et al, 1977). Later reports from Scandinavia and Britain, however, contended that there were no significant differences in plasma testosterone levels of BPH and age-matched healthy men, (Vermeulen, Rubens and Verdonck, 1972; Ghanadian, Lewis, Chisholm and O'Donoghue, 1977), while other investigators from Finland and Germany recorded higher testosterone levels in BPH compared with the levels in age-matched controls (Hammond, Kontturi, Vihko and Vihko, 1978; Bartsch, Becker, Pinkerburg and Krieg, 1979; Lukkarien, 1980).

Similar determinations of circulating levels of other androgens have also not been helpful in elucidating the role of plasma androgens in prostatic disease. Some studies reported higher plasma DHT levels in elderly BPH compared with healthy young men (Horton et al, 1975; Ishiamaru et al, 1977) and compared with age-matched healthy men (Vermuelen and De Sy, 1976; Ghanadian et al, 1977; Hammond et al, 1978; Bartsch et al, 1979). Other reports maintained that there is no significant difference in DHT levels between BPH and the healthy group (Habib, Lee, Stitch and Smith, 1976[b]; Lukkarien, 1980). Furthermore, plasma levels of A-Dione and 3αdiol have similarly not been found to be significantly different in BPH patients and healthy young men or age-matched controls (Habib et al, 1976[b]; Vermuelen and De Sy, 1976; Bartsch et al, 1979; Ghanadian and Puah, 1981).

Plasma Androgens in CaP

Though the clinical treatment and management of CaP is based on the concept of androgenic hormone dependence of

the tumour, determination of plasma androgen levels has not been helpful in assessing the endocrine milieu which may be conducive to the growth and spread of a primary tumour of the prostate.

Thus most of the studies did not show any significant difference in plasma testosterone levels between age-matched healthy men and untreated CaP patients (Moon and Flocks, 1970; Harper et al, 1976; Habib et al, 1976; Ghanadian and Puah, 1981).

Robinson and Thomas (1971) reported lower testosterone levels in patients with advanced stages of prostatic cancer. Furthermore, the detailed study by the British Prostate Study Group (1979) did establish that testosterone level is not a good discriminator of CaP, nor is it a good monitor of the progression of the tumour, since there is a considerable variability in plasma testosterone levels in both CaP patients and healthy controls. However, there appears to be consensus that administration of oestrogens to CaP patients lowers the plasma testosterone levels considerably (Robinson and Thomas, 1971; Editorial, Lancet, 1980).

Studies involving the determination of plasma DHT, A-Dione and 3α Diol have also reported similar results for both CaP patients and healthy men (Harper et al, 1976; Habib et al, 1976[b]; Hammond et al, 1978).

Therefore, from the above review, it appears that plasma androgens may not have direct effects on the development of prostatic disorders, though a permissive role could not be ruled out entirely.

Plasma Androgens and Prostatic Diseases in Nigerians

Following the reports which suggest low incidence of

prostatic diseases in Nigerians compared with other blackmen and Caucasians (Jackson et al, 1977; Ahluwalia et al, 1981), some attention has been focussed on the hormonal environment of the prostate of the Nigerian male, especially the circulating androgens (Ahluwalia et al, 1981). Reports from such studies showed that plasma testosterone levels in Nigerian healthy males are significantly lower than the levels in either Black Americans or Caucasians (Jackson et al, 1977; Ahluwalia et al, 1981). Furthermore, plasma testosterone levels in Nigerian CaP patients are not only lower than the levels in healthy males but are also lower than the levels in age-matched Black American CaP patients (Ahluwalia et al, 1981). It is significant that the levels of DHT are not different between the American and Nigerian patients and controls.

However, in the absence of any other reports of androgen levels in Nigerian BPH and CaP patients and healthy males above 50 years old, the speculation on the androgen status of Nigerian males vis-a-vis prostatic disorders, remains unproven. Though available reports suggest that plasma testosterone levels in healthy Nigerian males between 16 and 45 years are not significantly different from the levels reported for Caucasians (Dada and Nduka, 1980; Bolarinwa and Andy, 1982), nevertheless there is a need to re-examine the androgen status of the Nigerian males especially between 50 and 90 years, when the incidence of prostatic disorders could be expected to increase. This should involve the determination of plasma levels of testosterone, DHT, A-Dione and 3α diol in BPH and CaP patients compared with age-matched healthy males, with a

view to elucidating the role (if any) these androgens play in prostate pathology in Nigerians.

It is still a subject for debate whether peripheral plasma levels of hormones reflect the physiological state of the target tissue. This is why some believe that determination of intracellular concentrations of hormones is a more valuable means of elucidating the biological effects of these hormones and the physiological state of the prostate gland.

The Role of Intracellular Androgens in Prostate Pathology

Since available evidence suggests that circulating plasma androgens may not be the stimulant for the development of prostatic tumours (Habib et al, 1976(b); Wilson, 1980), it is logical to assume that certain changes might be initiated within the prostate gland itself.

Early indications that the hormonal environment in the prostate gland is unique came from results of animal experiments. Analysis of radioactive metabolities obtained one hour following administration of radioactive testosterone to castrated rats, showed over 90% as DHT in the prostate gland (Bruchovsky and Wilson, 1968) and the translocation of this metabolite into the nucleus within 2 hours has been recorded (Fang, Anderson and Liao, 1969).

Similar events have been shown to occur in human prostate with a high affinity binding of DHT in the gland (Hansson and Tveter, 1971). However, the suggestion that nuclear events in prostate gland are stimulated by DHT whereas androstanediols influence cytoplasmic processes (Ohno et al, 1971) appear to be an indication that the accumulation of these metabolites may be involved

in intraprostatic derangement. Therefore, the reported elevation of DHT level in hyperplastic prostate compared with normals and the observed 2-fold increased concentration in the periurethral area compared with outer areas of the gland (Siiteri and Wilson, 1970) generated interest and enquiries into the role of intraprostatic androgens in prostate pathology.

In this regard, many of the studies focussed attention on the determination of the concentrations of androgens in hyperplastic, malignant and normal prostatic tissues as a means of investigating the intraprostatic changes culminating in prostatic disorders. Many investigators confirmed higher DHT concentrations in BPH compared with either normals or malignant tissues (Habib et al, 1976[a]; Krieg, Bartsch, Janssen and Voigt, 1979). Geller, Albert, Lopez et al, (1976) found higher levels in older healthy subjects (65-84 years) than in those less than 40 years old, thus implying that DHT could also accumulate in a normal gland. The levels reported in these cases were, however, still much lower than those obtained in clinical hyperplasia.

Studies of DHT levels in Carcinoma tissues have shown lower concentrations than in BPH (Habib et al, 1976[a]; Geller, Albert, Lopez et al, 1978; Ghanadian and Puah, 1981). Higher DHT levels have been reported in carcinoma tissues by Hammond (1978), but Krieg et al (1979) did not find any significant difference between CaP and BPH tissues.

Low concentrations of testosterone have been reported for both BPH and normal prostatic tissues (Habib et al, 1976[a]; Hammond, 1978; Krieg et al, 1979) and higher levels in malignant tissues (Habib et al, 1976[a]; Hammond, 1978;

Ghanadian and Puah, 1981). However, Albert, Geller, Geller and Lopez (1976) obtained higher testosterone concentrations in BPH compared with carcinoma tissue. Levels of androstanediol (3α diol) were decreased in BPH compared with normals (Geller et al, 1976; Hammond, 1978; Krieg et al, 1979) while levels in carcinoma tissues were relatively higher (Hammond, 1978; Krieg et al, 1979; Ghanadian and Puah, 1981). Levels of prostatic androstenedione(A-Dione) were higher in carcinoma tissue than in BPH and normals (Habib et al, 1976[a]; Hammond, 1978).

Further insight into intracellular changes in the gland was provided by investigation of subcellular concentrations of androgen metabolites. Available reports showed that concentrations of DHT were higher in both nuclear and cytosol fractions of BPH tissue, whereas 3α diol levels were much lower (Meikle, Collier, Middeton, Fang, 1980). There were no data on the subcellular distribution of other metabolites in prostatic tissues.

Thus accumulation of DHT occurs commonly in BPH but its depletion is the rule in carcinoma. The low concentration of DHT in malignant tissue is replaced by high concentration of testosterone and A-Dione (Habib et al, 1976[a]). However, it is not known whether these features represent the consequences of intraprostatic derangement of androgen metabolism, or are the cause of these disorders. Whichever it is, determination of intracellular concentrations of androgens especially at the molecular level, should be a reflection of the hormonal environment of the gland, and could be valuable in identifying a diseased gland.

Therefore, in view of the reported low incidence of

prostatic disorders amongst Nigerians, it is pertinent to investigate the endocrine milieu of the prostate glands in this population in order to elucidate the role of androgens as a factor in these disorders.

Furthermore, since prostatic androgen concentrations are the results of androgen metabolism in the gland, a detailed study of the mechanisms of this metabolism could provide the explanations for the different androgen concentrations observed in pathological prostates.

Androgen Metabolism and Disorders of the Prostate

Accumulated evidence over the last two decades has shown conclusively that for maximum physiological activity at the target tissue, testosterone must be metabolized to several closely related metabolites, among which DHT is of outstanding importance (Fig. 1.4 page 23) (Ofner, 1970). Early studies of this metabolism demonstrated that testosterone is enzymatically converted in human prostate to 5α(β) androstane derivatives (Pearlman and Pearlman, 1961) while Farnsworth and Brown (1963) showed that the conversion was principally to DHT and 5α androstane, 3α 17β diol (3α diol). The enzymes involved in these transformations were later identified and characterized as 5α(β) reductases, 3α(β) hydroxy steroid dehydrogenases (an oxidase-reductase) and 17β hydroxy steroid dehydrogenase (Chamberlain, Jagarinec and Ofner, 1966). Thus, it is now known that in human prostate, testosterone diffuses into the prostatic cell where it is irreversibly converted by 5α reductase to DHT. The DHT so formed is partly complexed to a cytoplasmic receptor-protein and partly further metabolised by 3α(β)-OHSD to 3α(β) diol. The DHT-receptor

complex is translocated to the nuclear membrane, where it is also attached to a nuclear-receptor, prior to its being bound to chromatin for appropriate biological process. Some testosterone is also oxidised by 17β OHSD to A-Dione (Fig. 1.4 page 23).

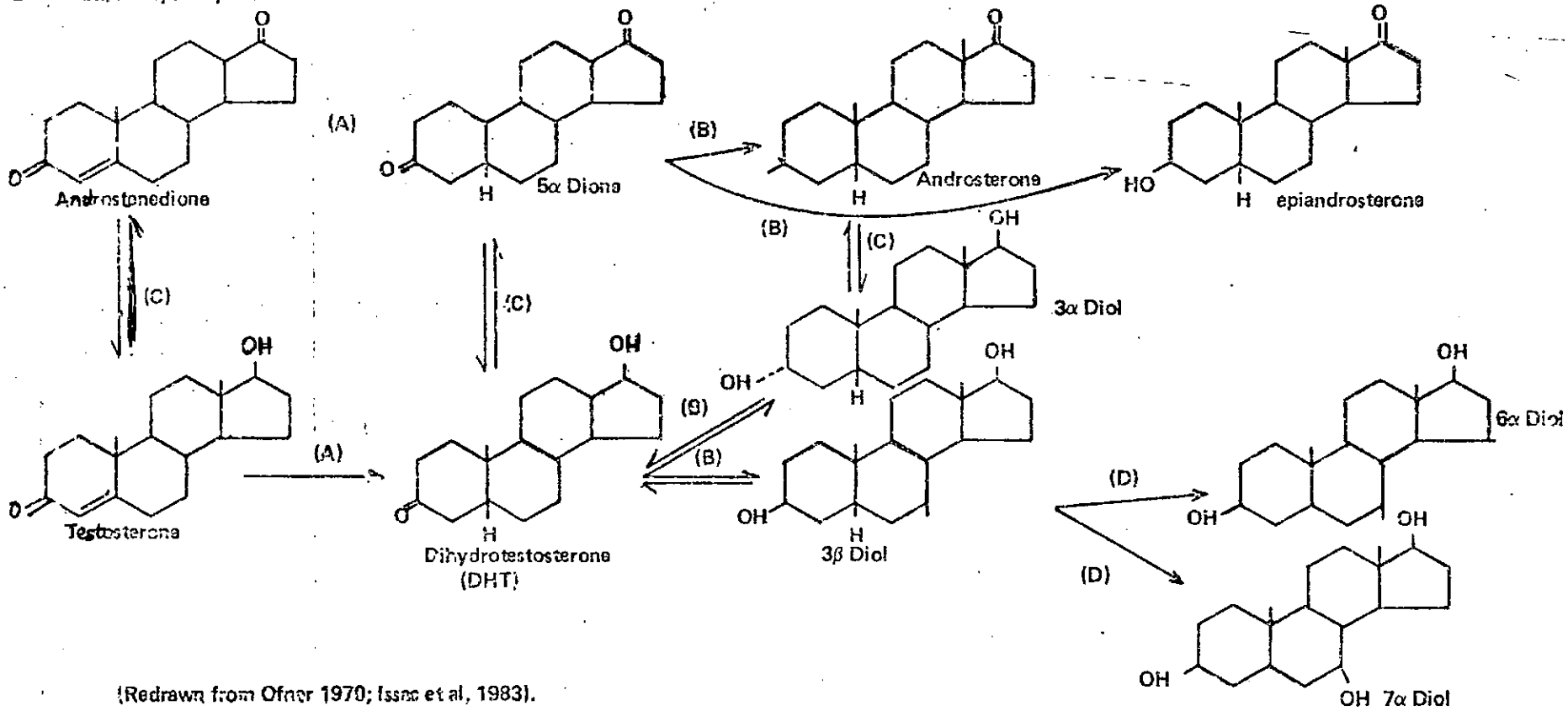
It has been suggested that any derangement in the above metabolic processes may result in accumulation or depletion of androgen content of the gland and that this may cause disorders. Hence research efforts have been focused on the understanding of the mechanism of the metabolic processes. Most of the studies in humans have involved in vitro experiments, using prostate tissue homogenates and slices from normal, BPH and CaP patients, with various androgens as substrates. However, most of the results obtained have neither been consistent with the results of intracellular androgen concentration in the same tissue, nor conclusive in explaining the role of androgen metabolism in prostate pathology.

Therefore, the question has been asked whether accumulation or depletion of androgens in prostate gland is a consequence of increased synthesis or degradation promoted by increased or decreased enzyme activities. Hence, the activities of these enzymes especially at the molecular level have been studied by many investigators.

FIG. 1.4 METABOLIC PATHWAY OF ANDROGENS IN HUMAN PROSTATE

KEY TO ENZYMES

A -- 5α reductase
B -- 3α(β) OHSD (Oxido–Reductase)
C -- 17β OHSD (Oxido–Reductase)
D -- 6α/7α Hydroxylase



(Redrawn from Ofner 1970; Issa et al, 1983).

5α reductase activity

This enzyme is responsible for the initial conversion of testosterone to DHT in the cytosol and nucleus of prostatic cell. Earlier studies comparing normal and hyperplastic tissues did not record any differences in 5α reductase activity in the tissues (Shimazaki, Kurihara, Ito and Shida, 1965; Siiteri and Wilson, 1970; Prout, Kliman, Daly et al, 1976; Jenkins and McCaffery, 1974), despite the observation that hyperplastic tissue contains high DHT concentration (Siiteri and Wilson, 1970; Habib et al, 1976[a]; Krieg, Bartsch, Herzer et al, 1977). However, more intensive studies have now established the fact that high concentrations of DHT in hyperplastic prostate are caused by increased synthesis mediated by increased 5α reductase activity (Djoseland, Tveter, Attramadal et al, 1977; Morfin, Distefano, Bercovici and Floch, 1978; Bruchovsky and Liekovsky, 1979; Isaac et al, 1983). With regards to malignant gland, results have been less conclusive. Some studies reported low 5α reductase activity (Habib et al, 1979[a]; Krieg et al, 1979), even though relatively high DHT concentrations have been obtained in such tissues (Hammond et al, 1978; Bruchovsky and Liekovsky, 1979). Krieg et al (1979) did not observe any significant differences in DHT concentrations between BPH and CaP despite the low 5α reductase activity in CaP tissues. Therefore, it appears that accumulation or depletion of DHT in the prostate gland may not be due to 5α reductase activity alone, but may be influenced by other factors, such as the concentrations of trace-metals in the gland.

3α OHsD Activity

DHT is further metabolised to 3α(β) diols, through the activity of 3α(β) Hydroxysteroid Dehydrogenase (OHsD). Therefore the activity of this enzyme could influence the concentration of DHT in the gland. Studies by Bruchovsky and Liekovsky (1979) and Hudson (1982) have reported low 3α(β) OHsD activity in hyperplastic tissue. These results are consistent with high DHT concentrations found in this tissue (Geller et al, 1976; Ishiamaru et al, 1977). In contrast, some reports maintained that the activity of the enzyme remains high in BPH tissue (Farnsworth, 1972; Habib et al, 1979), while others could not demonstrate any differences in activity between normal and BPH tissues (Shida, Shimazaki, Ito et al, 1975).

In malignant tissue, 3α OHsD activity was low (Bruchovsky and Liekovsky, 1979; Habib et al, 1979[a]).

17β OHsD Activity

The role of this enzyme in prostatic disorders has remained relatively uninvestigated. It may be important in the prevention of DHT accumulation in prostate - a common feature in BPH gland. Available evidence now suggests the predominance of 17β OHsD activity in malignant gland, resulting in the accumulation of testosterone and A-Dione in such glands (Habib et al, 1976[a]; Hammond et al, 1978; Krieg et al, 1979). This observation is suggestive of increased 17β OHsD activity in malignant gland compared to BPH, though increased activity has also been reported in normal tissue (Morfin et al, 1978). The reason for the latter observation is not clear but may be due to decreased activities of 5α reductase and 3α OHsD.

Subcellular Activities of Enzymes

Most of the experiments reviewed above employed prostatic tissue homogenates, minces and slices, whereas there appears to be a localisation of some of the enzymes in a particular subcellular fraction of the prostatic cell. Therefore, further studies involving isolated subcellular fractions have been undertaken, in order to obtain greater insight into the mechanism of androgen metabolism in human prostate.

Though 5α reductase is particularly active in cytosol where the primary conversion of testosterone to DHT takes place, a nuclear 5α reductase has been isolated (Wallace and Grant, 1975) even though its role *in vivo* in this fraction is less certain. Similarly, it now appears that 3α OHsD which has been regarded as predominantly a cytoplasmic enzyme (Jacob and Wilson, 1977) is also present in the nucleus, as earlier postulated by Belham, Necl, William (1969). This view was reinforced by the determination of nuclear 3α diol concentration in both normal and BPH tissues (Meikle et al, 1980). Hudson (1982) did not observe any 3α OHsD activity in the nuclear fraction of either normal, BPH or CaP tissue; a finding which brings into question the origin of the 3α diol reported earlier.

In vitro 5α reductase activity in both nuclear and supernatant fractions showed conversion of testosterone to predominantly DHT (Grant, Minguell, Taylor and Weiss, 1971; Habib, Rafati, Robinson and Stitch, 1979). This corresponds to the findings with whole tissue homogenates (Krieg et al, 1979). With 3α OHsD and 17β OHsD, the situation is less clear. Jacob and Wilson (1977) and Morfin et al (1978)

reported increased 3α OHsD in both cytosol and microsomes of BPH tissue, while in contrast, Hudson (1982) obtained much lower activity in cytosol and microsome of BPH compared to normal and malignant tissues. In spite of these, these enzymes appear to play important roles in the prostate gland.

Current hypothesis is that prostatic hyperplasia is enhanced by various enzymatic interactions favouring accumulation of DHT in the gland (Isaac et al, 1983). Recent in vivo experiments have also shown that decreased conversion of DHT to 3αdiol may be promoting hyperplasia in elderly men (Morimoto, Edmiston and Horton, 1980). The concept that DHT formed in the cytoplasm is translocated into the nucleus suggests that high nuclear concentrations of DHT may be causal to prostatic disorders. Nuclear DHT is further transformed through either a reductive (3α diol formation) or oxidative (DHT formation from 3α (β) diol) pathway. This presumes there is a 3α (β)OHsD (oxidoreductase) in the nucleus, which induces nuclear DHT reduction but can also increase formation when its oxidative characteristics predominate (Morimoto et al, 1980; Isaac et al, 1983). This postulate would become untenable if the reported absence of nuclear 3αOHsD, even in normal tissue (Hudson, 1982) were valid.

The sum total of this review is to show that the above concept of the intraprostatic interaction which may result in prostatic disorders, requires further detailed investigation, especially at the molecular levels. Thus, it may be relevant to study:

i. the conversion of testosterone to DHT;

ii. the conversion of DHT to 3αdiols;

iii. the conversion of 3α (β)diol to DHT in whole tissue homogenate, nuclear and cytosol fractions of normal, BPH and CaP tissues, in order to elucidate the role of enzymatic transformation of testosterone in the development of prostatic disorders.

Zinc and Cadmium in Prostate Pathology

Besides androgens, other biochemical factors like trace-metals, especially zinc and cadmium, are considered to be involved in prostatic disorders.

Zinc in Human Prostate

Bertrand and Vladesco (1921) observed high concentrations of zinc in human prostate. Their study of zinc content of various human organs showed that apart from the semen, the prostate contained the highest zinc levels. Mawson and Fischer (1952) confirmed the presence of high zinc concentrations in the prostate and also suggested variations in zinc concentrations within the same gland. Kerr, Keresteci and Mayoh (1960) made similar observations and added that there was a tendency for zinc levels to increase towards the caudal end of the gland. Attempts to eliminate within gland variations in zinc content by determining the concentrations in the whole gland instead of a representative portion resulted in higher values than previously reported (Leissner et al, 1980).

Distribution of zinc within the prostatic cell showed histochemical localization in both nucleus and cytoplasm (Mager, McNary and Lionett, 1953). Histochemical findings of scanty staining in cytoplasm of malignant tissue and

intense staining in hyperplastic tissue, appear to agree with chemical values of zinc levels in these tissues (Gyorkey, Minn, Huff and Gyorkey, 1967). Similar results of elevated zinc levels in hyperplastic glands and low levels in malignant glands were also reported by Schdrodt, Hall and Whitemore in 1964.

Habib, Hammond, Lee at al (1976a) did not observe any significant difference in zinc concentrations in normal and hyperplastic prostates. There were other reports showing higher zinc concentrations in BPH gland than in healthy controls and in malignancy (Gonick, Oberleas, Kneehtges, Prasad, 1969; Jaffa, Mahendra, Chowdhury and Kambos, 1980; Leissner et al, 1980; Feustel, Wennrich, Steeniger, Klauss, 1982).

Zinc and Androgens in Prostate

Although the human prostate contains very high concentrations of zinc, the physiological function of the zinc is still obscure. Many investigators have studied the relationship between zinc and androgens in the prostate, since the prostate is an androgen-dependent gland. Results from animal experiments provided data on the relationship. Gunn and Gould (1956) observed increased ^{65}Zn uptake by the prostate of castrated rats after administration of testosterone. Similar results were also obtained in canine prostates where absence of testicular hormone significantly altered radioactive zinc uptake (Prout. Sierp and Whitemore, 1959; McKenzie, Hall, Whitemore, 1963)

However, the relationship between zinc and androgens has not always been evident in human prostate. Studies by Habib et al (1976a) with transurethral sections of normal,

hyperplastic and malignant prostates showed no correlation between testosterone and zinc in any of the tissues, and the authors, therefore, concluded that the uptake of testosterone in hyperplastic tissue is independent of the metal concentration. Habib, Mason, Smith and Stitch in 1979 suggested a strong association between DHT : T ratio and zinc in both BPH and CaP tissues. They proposed the use of this relationship as an index for predicting the onset of neoplasm in prostate glands. More recently, zinc concentrations in prostate glands of prepubertal boys were found to be of similar concentration as those of non-prostatic tissue; a finding which suggested that the increased zinc concentrations found in adult prostates could be due to the influence of androgen stimulation (Leissner et al, 1980).

Subcellular distribution of androgens in BPH showed a higher concentration of DHT in the nuclear and cytosol fractions (Meikle et al, 1980). Similarly, zinc has also been shown to be distributed between the nucleus and cytoplasm (Reed and Stich, 1973; Leake, Chisholm, Busuttil and Habib, 1984). There are no reports relating the quantitative levels of zinc to the subcellular concentration of other androgens.

Zinc and Androgen Metabolism in Prostate

The physiological functions of the endogenous prostatic zinc was thought to include the modulation of the conversion of testosterone to other metabolites by the prostate gland. This has been investigated through *in vitro* incubation studies involving various androgen substrates and the addition of varying concentrations of exogenous zinc. Grant et al (1971) reported the complete

inhibition of the formation of DHT from testosterone, in the nuclear fraction of hyperplastic tissue, by additions of 50umol of exogenous zinc. In contrast, additions of lower concentrations of exogenous zinc appears to enhance the formation of DHT by between 20% and 30%. These findings suggest that nuclear 5α reductase is susceptible to high concentrations of zinc. Wallace and Grant (1975) confirmed the inhibition of the formation of DHT, but could not demonstrate stimulation of the conversion by low concentrations of zinc reported earlier.

There appears to be a significant inverse relationship between Zn^{++} concentration and 5α reductase activity (Wallace and Grant, 1975).

In a more elaborate study, Habib (1978) reported the in vitro effect of addition of varying concentrations of zinc on the formation of DHT from testosterone, by whole tissue homogenates of hyperplastic tissues. This study showed that conversion of testosterone to DHT was suppressed by exogenous zinc concentrations greater than 10^{-5}M (10umol) while concentrations between 10^{-6} and 10^{-8}M enhanced DHT formation. These findings are at variance with those of Wallace and Grant (1975). An interesting feature of the report by Habib (1978) is the inhibition of DHT formation by very low concentrations of exogenous zinc ($< 10^{-8}$), though the degree of inhibition was not stated.

Since high concentrations of DHT have been found in the nuclear (Meikle et al, 1980) and whole tissue homogenates of hyperplastic prostate (Siiteri and Wilson, 1970; Geller et al, 1976; Krieg et al, 1977), the above in vitro experiments suggest that some level of zinc is required for

the formation of DHT. There may therefore be a physiological role for zinc in the endocrine mediated disorders of the prostate.

It is not certain whether these in vitro findings reflect in vivo situation in the gland. This doubt is borne out by the recent report by Sinquin, Morfin, Charles and Floch (1982) that endogenous zinc levels have no effects on the 5∝reductase activity in prostate. The finding may be due to the very low concentrations of zinc in the tissues (33.0 - 181.5 ug/g) and the diminished 5∝reductase activity. In any case, it is obvious that the hypothesis that zinc is involved in the endocrine disorders of prostate requires further detailed study involving both endogenous and exogenous concentrations of zinc.

Zinc and 3∝OHsD Activity

The role of endogenous zinc in the metabolism of DHT to 3∝(β)diol is also not known for certain. Evidence from animal experiments suggests that zinc can inhibit the formation of 3∝diol in the rat ventral prostate (Mawhinney and Belis, 1976). Studies in human prostate have been less conclusive. Morfin et al (1978) obtained an increased 3∝reductase activity on the addition of 1mmol of exogenous zinc to whole tissue homogenate of hyperplastic tissue, but this effect was absent when the experiments were repeated with microsome and cytosol fractions. These findings have been partly supported by Hudson (1982) who also could not demonstrate any effect of low concentrations of exogenous zinc (2umol) on the prostatic cytosol 3∝OHsD activity.

Addition of 2mM caused a 30% inhibition. Similar findings have been reported for endogenous concentrations of

zinc where 0.2mM concentration caused the inhibition while a lower concentration (0.05mM) enhanced the formation of 3∝diol in hyperplastic tissue (Sinquin et al, 1982).

In view of the inconsistency in the available reports, the effect of zinc on 3∝OHsD activity, especially at the molecular levels, requires more detailed investigations.

Cadmium and Prostate Pathology

Suggestions that cadmium may be a factor in the aetiology of prostatic disorders were first made by Kipling and Waterhouse in 1967. They reported increased incidence of CaP in a group of workers exposed to cadmium oxide. However, other epidemiological studies could not confirm this association between cadmium and carcinoma of the prostate (Lemen, Lee, Wagoner and Blejer, 1976; Kolonel and Winkelsten, 1977). Therefore, other workers resorted to animal experiments where attempts were made to induce prostatic tumour via cadmium administration. Even in these cases, results were not conclusive.

The toxic effects of administration of high concentrations of cadmium on rat prostate have been successfully demonstrated (Timms, Chandler, Morton and Groom, 1977; Khore, Der, Ross and Fahim, 1978). Administration of low concentrations failed to produce any ultrastructural changes; nor could any prostatic adenocarcinoma be induced (Battersby, Chandler, Morton, 1982). These pieces of evidence suggest that the role of cadmium in prostate pathology remains unknown. Nevertheless, an earlier suggestion by Parizek (1957) of a competitive inhibition between zinc and cadmium in rat testis has continued to stimulate research into the relationship between zinc and cadmium in other male

sex accessory organs. The possibility of this relationship appeared confirmed in the prostate by Aughey, Scott, King et al (1975) who reported that areas of prostate with high zinc concentrations retained less cadmium, while cadmium accumulated in the areas with low zinc concentrations. Studies in human prostate have also shown increased cadmium concentrations in malignant tissues following depletion of zinc in such tissues (Habbib et al, 1976(a); Feustel et al, 1982).

Since zinc has been linked as an aetiological factor in prostatic disorders, attempts have been made to investigate the involvement of cadmium. Reed and Stitch (1973) have shown that cadmium reduces the uptake of zinc by prostatic tissue, and inhibits in vitro conversion of testosterone to DHT (Wallace and Grant, 1975).

Therefore, it appears that cadmium, too, may be directly or indirectly linked to the development of benign and malignant disorders of the prostate, through yet unknown mechanisms.

Plasma Zinc, Cadmium and Prostate Pathology

It is generally believed that the main source of intraprostatic zinc and cadmium is the peripheral blood, but whether the blood levels mirror the tissue concentration remains controversial (Willden and Robinson, 1975; Kjellstrom and Nordberg, 1978; Habib, Dembinski and Stitch, 1980; Jaffa et al, 1980). Many investigators over the years have observed changes in the levels of zinc in plasma and cellular elements in various diseases. However, these changes have not been consistently observed in diseases of male sex accessory organs despite the reported high zinc

concentrations in these organs.

Willden and Robinson (1975) recorded significantly higher zinc levels in BPH compared with age-matched healthy subjects and lower levels in CaP relative to BPH. These findings were not confirmed by Habib et al (1980) who observed no significant differences in zinc levels between normals, BPH and CaP. In contrast, to above reports, Jaffa et al, (1980) not only maintained that plasma zinc levels were elevated in BPH and decreased in carcinoma, but also suggested that these levels mirror the tissue concentrations of the metal.

Furthermore, the possibility that elevated zinc levels in patients with confirmed prostate enlargement excludes the presence of malignancy, has been proposed (Willden and Robinson, 1975).

With regards to cadmium, it is known that this non-essential trace-metal is virtually absent from the human body at birth. It accumulates in the tissue (Anke and Scheider, 1971) and blood (Pleban and Pearson, 1979) continuously with age up to 50 years when the total body concentration (in an unexposed person) may reach 20-30mg (Underwood, 1977). Many studies have reported low cadmium levels in normal blood (Willden, 1973; Cernick-Sayers, 1975), but Kjellstrom and Nordberg (1978) have postulated that blood cadmium levels may not only be a reflection of the daily intake, but may also reflect the endogenous tissue level. Little information is available on plasma or blood levels of cadmium in prostatic disorders, though Habib et al (1976) have reported elevated prostatic concentrations of cadmium in carcinoma of the prostate.

Plasma zinc levels in healthy Nigerians are similar to the levels in Caucasians (Olatunbosun, Akindele, Adeniyi and Bademosi, 1978; Ette, Ofodile and Oluwasanmi, 1982) but levels are decreased in some diseases (Olatunbosun et al, 1978). However, there are no reports of plasma zinc and cadmium in Nigerians with prostatic disorders. Therefore, investigation of plasma levels of these metals may not only provide basic data for Nigerians but may also add to our knowledge of prostate pathology.

Hypothetical Concept of the Roles of Androgens, Zinc and Cadmium in the Development of Prostatic Disorders

As already indicated, it is possible that zinc and/or cadmium may have physiological roles in the pathogenesis of disorders of prostate, though the actual mechanism is as yet unknown precisely.

Review of different proposals by many investigators reveals that some areas require more detailed investigation. Therefore, a hypothetical concept of the roles of these metals is being proposed and investigations of this hypothesis form the central theme of this study. The hypothesis is based on the following premise (Fig. 1.5, page 37).

Available blood zinc and cadmium diffuse into the prostatic cell where they are concentrated in both the cytoplasm and nucleus, thus creating a blood-tissue gradient in a sort of dynamic state. In normal metabolic process, intraprostatic zinc promotes the conversion of testosterone to DHT in the cytoplasm and DHT to Diols in both cytoplasm and nucleus of the prostatic cell, thereby preventing accumulation of DHT and the attendant hyperplasia. However, if the blood-tissue gradient changes in favour of the tissue, the resultant high zinc concentration could initiate any of two reactions.

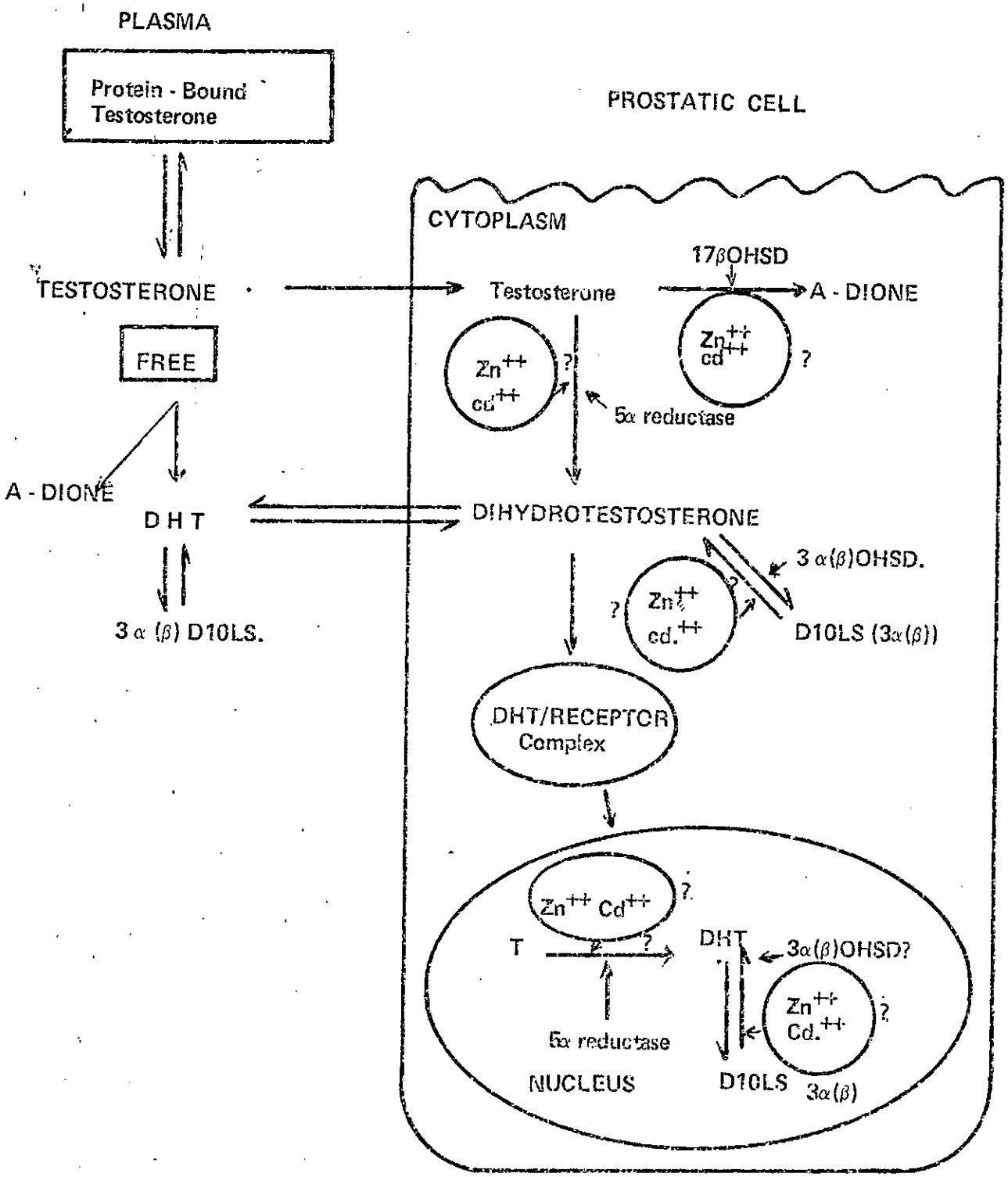


FIG 5 Diagramatic Concept of the roles of Zinc and Cadmium in modulating the metabolic processes in the prostate cell.

1. If the tissue zinc is concentrated predominantly in the nucleus (see Reed and Stitch, 1973; Leake et al, 1984) this could inhibit further metabolism of DHT to Diols in the nucleus. Meanwhile, the normal process in the cytoplasm continues to augment the DHT available to the nucleus. These reactions could result in accumulation of DHT in the nucleus, the consequence of which may be the initiation of hyperplastic process.

2. Concentration of zinc mainly in the cytoplasm could inhibit the conversion of testosterone to DHT to 3αdiol. This blockage could result in accumulation of testosterone and eventual depletion of nuclear DHT. It is possible that persistence of such a situation could initiate neoplastic changes.

On the other hand, the alteration of blood-tissue gradient in favour of blood could result in the depletion of intraprostatic zinc. This could affect, adversely, the normal process of DHT and 3αdiol formation in cytoplasm and the nucleus, thus promoting excessive concentration of testosterone and A-Dione which could initiate neoplastic changes in the cell. Consequent to the depletion of intracellular zinc, cadmium could diffuse increasingly into the cell and be concentrated in both nucleus and cytoplasm. The continuation of such a situation could also aid neoplastic changes in the cell. Admittedly, this hypothesis may be partly tenable or completely invalid, nevertheless investigation of each stage would enhance the present knowledge on the development of prostatic disorders. The outline of these investigations are stated as follows:

1.3. OUTLINE OF THE PRESENT STUDY

Several studies have investigated various biochemical factors and processes which may be involved in the development of benign hyperplasia and malignancy of the prostate. This study was designed to investigate the contributions of endocrine control of the prostate and the physiological roles of zinc and cadmium in the aetiology of these disorders in Nigerians. Since biological expression of a cell is exhibited at the molecular level, knowledge of the mechanism by which these tissue-metals and androgens interact at the molecular level appears to be essential for the understanding of their roles in the pathogenesis of BPH and CaP.

Therefore, this study was specifically focused on the following areas:

1. Investigations of the endocrine control of the prostate of healthy subjects and patients with BPH and CaP, by:-
   - i. The determination of plasma levels of androgens in the above groups within the age group 50 - 90 years.
   - ii. The determination of prostatic tissue concentrations of androgens in the same groups of patients and in normals (autopsy specimens).
   - iii. The determination of androgen concentrations in the subcellular fractions of prostatic tissues from the same group as above.
2. Investigations of the roles of plasma trace-metals in the endocrine control of the prostates of healthy

subjects and in BPH and CaP, by:

i. The determination of plasma zinc and cadmium in the three groups of subjects.

3. Investigations of the relationship between androgens and trace-metals in prostatic disorders, through:

i. The determination of zinc and cadmium in prostatic tissues from healthy subjects, BPH and CaP patients.

ii. The determination of these trace-metals in the subcellular fractions of prostatic tissues.

iii. In vitro experimental studies of androgen metabolism in prostatic tissue homogenates.

iv. In vitro experimental studies as above in the subcellular fractions of prostatic tissues.

4. Investigations of the roles of zinc and cadmium in the metabolism of androgens by the prostate:

i. In vitro experimental studies of the effect of zinc and cadmium on the above metabolism.

C H A P T E R 2

MATERIALS AND METHODS

"Nothing is as easy as it
looks,
Everything takes longer than
you expect,
And if anything can go wrong
it will,
At the worst possible moment!"

Murphy's Law.

2.1. MATERIALS

2:1.1 Chemicals and Reagents

(i) Ethylene diamine tetraacetic acid (EDTA)-2 mM in Tris-Hcl buffer pH 7.4.

(ii) Glycerol - 10% v/v

(iii) NADPH - 0.5 mM in Tris-Hcl buffer

(iv) Phosphate - Saline buffer 0.1M - 10.196g Sodium hexametaphosphate in 1 litre of 0.85% NaCl.

(v) Gelatin - 0.1%

(vi) Sodium merthiolate 0.01%

(vii) Dextran coated charcoal ("Separex" tablets - Steranti Research Limited). Each tablet is equivalent to 250mg of Charcoal and 25mg of Dextran in 100ml of buffer.

(viii) Scintillator fluids (a) Butly PBD(-PPO)4g/l in Toluene : methanol (10:1)

(b) Triton x (Cocktail) contained

PPO - 15g

POPOP - 1g

Triton x - 1250ml.

Xylene - 3250ml.

(ix) Zinc chloride 1g/l

(x) Cadmium chloride - 0.5mol/l

(xi) Tris-Hcl buffer - 0.05M

Tris base 6.057g/l adjusted to pH 7.4 with HCl.

Buffer also contained 10ml Glycerol (10%v/v)

2:1.2 Organic Chemicals

All organic chemicals used are of "Analar grade" and are re-distilled when necessary to maintain purity.

2:1.3 Non-radioactive Steroids

100mg/100ml in ethanol of each of the following non-radioactive steroids were prepared: Testosterone, Dihydrotestosterone, Androstenedione and Androstanediol 3α and Androstanediol 3β, 5α Androstanedione and Androsterone.

2.1.4 Radioactive Steroids

(1,2,6,7 ^{3}H) Testosterone, Sp. Act. 80-105Cl/mmol

(1.2.4.5,6,7, ^{3}H) 5αdihydrotestosterone, Sp. Act. 147 Cl/mmol

(1.2.6.7, ^{3}H) Androstanedione Sp. Act. 110 Cl/mmol

(1&2 (n) ^{3}H) 5α Androstanediol (3α 17β) Sp. Act. 60 Cl/mmol

(1&2 (n) ^{3}H) 5α Androstanediol (3α 17β) Sp. Act. 60 Cl/mmol

Above Stock Solutions were purchased from Radiochemical Centre, Amersham, England and were diluted appropriately to produce the required radioactivity for different procedures.

2.1.5 Antisera

All antisera used in this study were purchased from Steranti Research Limited, London, as freeze-dried material and were reconstituted with buffer to 1 : 100 dilution according to the

instructions of the manufacturers.

2.1.6 Special Reagents

Zinc chloride (anhydrous) The following concentrations were made in Tris-HCl buffer (7.4) 10^{-}M, 10^{-2}M, 10^{-4}M, 10^{-6}M, 10^{-8}M, 10^{-10}M, 10^{-12}M. Cadmium chloride - 10^{-1}M, - 10^{-12}M were made in Tris-HCl buffer (pH 7.4).

2.1.7 Chromatographic Materials

Sephadex LH - 20

Silica gel chromatographic plates 20 x 20cm.

2.1.8 Steroid Standards (Stock) contain 10ng/100ml of each steroid in ethanol, and were purchased from Steranti Research Limited, London.

2.1.9 Working Standards - prepared by dilution of 100ul of Stock in 10ml acetone, to contain 1pg/ul.

2.2. METHODS

2.2.1 PRELIMINARY EXPERIMENTS

2.2.1(a) Purity Check of Radioactive Steroids

The radioactive steroids ^{3}H Testosterone, ^{3}H DHT, ^{3}H Androstenedione, ^{3}H Androstanediol (3α) and ^{3}H Androstanediol (3β) used during the course of this study were checked quarterly for purity. Aliquots (100ul) of working solutions of the steroids were chromatographed on TLC alongside non-radioactive standards (Markers) in the system chloroform : ether (7:3). The spots were located by U.V. light absorption at 240nm or by exposure to iodine vapour in a glass jar. Spots were marked and then scooped into counting vials to which 5ml of Scintillator had been added. The radioactivity of each spot was counted and compared with the radioactivity in 100ul of same steroid (Total radioactivity). The percentage radioactivity recovered was calculated from:

$$\frac{\text{Recorded cpm of t.l.c. spot}}{\text{Recorded cpm of "Total"}} \times 100$$

The steroid was considered pure enough for use for subsequent experiment if:

(i) The steroid exhibited one spot only on the t.l.c.

(ii) The percentage recovery was not below 90%.

Results: The average percentage recovery obtained were as follows:

$[^{3}H]$ Testosterone 95%; $[^{3}H]$ DHT 92.5%; $[^{3}H]$ A-Dione, 98%; $[^{3}H]$ 3α Diol, 94%; $[^{3}H]$ 3β Diol, 94%.

2.2.1(b) Separation of Androgens on Column Chromatography

Separation of steroids can be accomplished in many different ways including solvent partition, paper chromatography, thin-layer chromatography and column chromatography. Since androgen steroids have small structural differences (Fig. 2.1, page 50), the assay of each androgen in a biological sample would require specific antiserum, to eliminate cross-reactions. Even then, purification of the extracts of the specimens would be required prior to radioimmunoassay of the specific androgen.

However, a less difficult approach is to separate individual androgens by any of the techniques mentioned above.

In this study, column chromatography using Sephadex LH-20 was used for the separation because of its many advantages over other methods, namely:

(i) High recovery

(ii) Good reproducibility

(iii) Sephadex LH-20 can be used without prior activation and can be washed and re-used.

(iv) Large amounts of biological fluids can be processed.

(v) Low blanks are obtained.

Therefore, the separation of plasma and tissue androgens was undertaken under the following specifications:

(i) Sephadex LH-20 - 0.9g/column

(ii) Solvent-System - Heptane : Chloroform:methanol (85 : 15 : 5)

(iii) Column specification - 20 x 0.7cm (i.d)

(iv) Effective column height - 70mm.

(v) Flow rate - 50ml/h.

Method:

0.9g of Sephadex LH-20 was soaked in 10ml of solvent-system overnight. This was replaced with fresh 10ml of solvent with which the gel was packed into the column to a height of 70mm. To each of five freshly prepared columns was added 100ul of each of the tritiated androgens (5000cpm) plus 50ug of each unlabelled androgen. Androgens were eluted from each column with about 50ml of solvent and 1ml. fractions were collected. Each fraction was put in a counting vial and evaporated to dryness. Radioactivity associated with each fraction was counted in 5ml. of Scintillator solution on the Tricarb Scintillator counter (Parkard) Model 3375. The cpm was plotted against fraction number on a graph.

With this procedure, the pattern of separation of androgens on Sephadex LH-20 was established.

Results:

The pattern of separation of androgens showed that A-Dione was eluted in fractions 1 - 7 with peak at 3 - 6, followed by DHT at fraction 10 - 13. Testosterone was contained in fractions 16--19 and 3αandrostanediol and 3βandrostanediol at 20 - 22 and 24 - 28 respectively (Fig. 2.2, page 51).

Having established this pattern, subsequent separation of plasma and tissue androgens prior to RIA were

undertaken by the above procedure. The "peaks" of fractions were pooled and employed as the sample for androgen estimation.

2.2.1(c) Establishment of Protocol for Radioimmunoassay of Androgens

The method of choice for the estimation of androgens in plasma and prostatic tissue was radioimmunoassay technique (RIA).

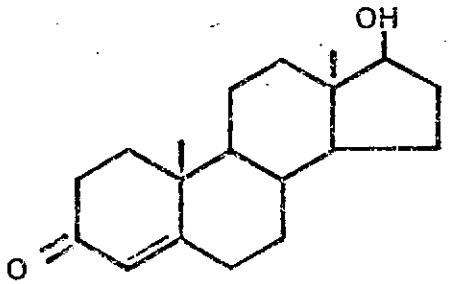
Procedure:

(i) Preparation of Plasma Samples

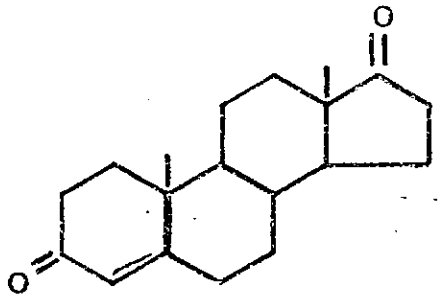
Androgens were initially extracted from 0.5ml of plasma with redistilled diethly ether (2 x 3mls), followed by column chromatographic isolation of individual androgens. The extract of each androgen was reconstituted in 0.5ml of buffer, from which 2 x 0.1ml were taken for RIA.

(ii) Preparation of Tissue Samples

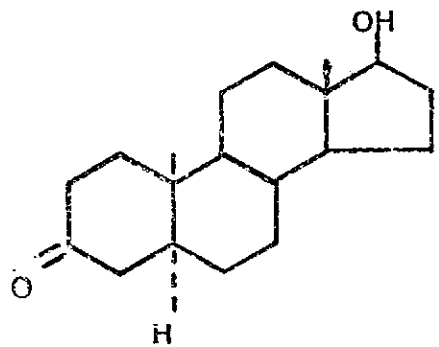
Prostatic tissue (0.5 - 0.8g) were first homogenised in distilled water, followed by extraction with redistilled dichloromethane.



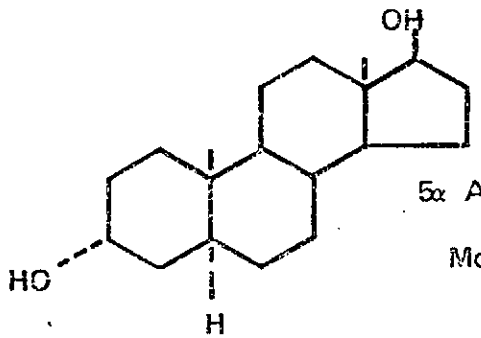
TESTOSTERONE
Mol. Wt. – 288.4.



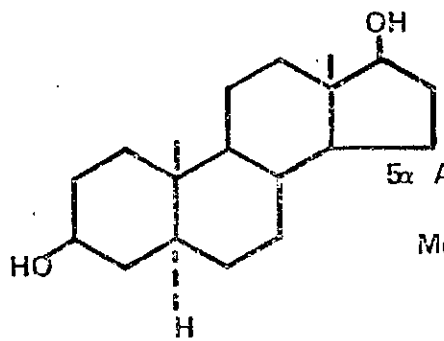
ANDROSTENEDIONE
Mol. Wt. – 286.4



5α DIHYDROTESTOSTERONE
Mol. Wt. – 290.4



5α ANDROSTANEDIOL
3α 17β
Mol. Wt. – 292.5



5α ANDROSTANEDIOL
3β 17β
Mol. Wt. – 292.5

Fig 2:1

FIG. 6 ANDROGENS INVESTIGATED IN THIS STUDY SHOWING STRUCTURAL AND MOLECULAR DIFFERENCES.

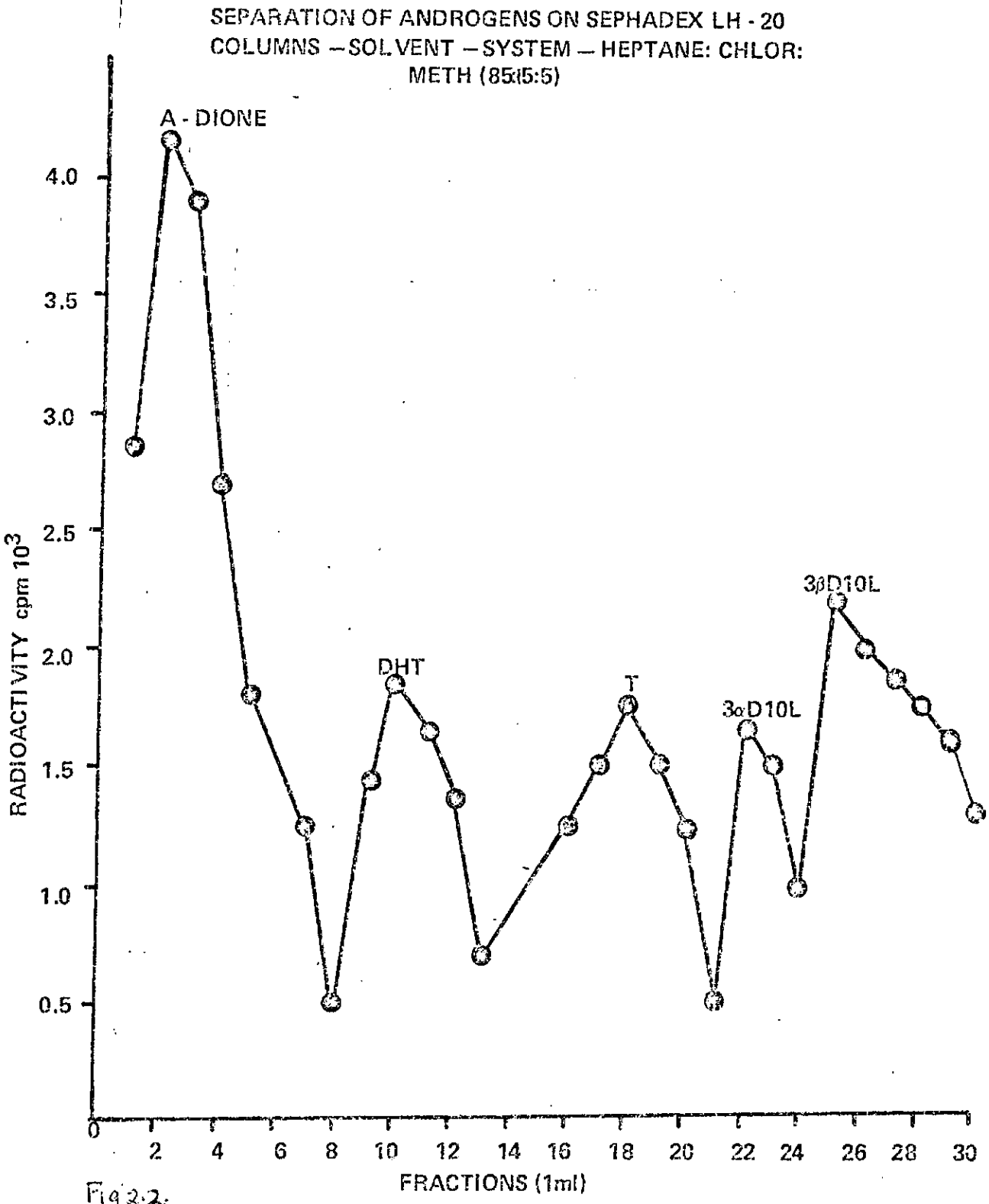


Fig 2.2.
FIG. 2 Elution pattern of androgens separated on Sephadex LH - 20 on 20 x 0.7cm (1:d) micro columns. Total gel used was 0.9g and effective column height 70mm.

The extract was dried and reconstituted in 0.2ml of solvent. Individual androgens in the extract were isolated by column chromatographic separation. Each chromatographic extract was dried and reconstituted in 0.5ml buffer, from which 2 x 0.2ml were taken for RIA.

(iii) Preparation of Standard Curve

Working standards containing 10,20,50,100,200,300 and/or 500pg of each androgen were prepared from working standard (100pg/100ul) (See 2.1.8 Materials) and put into test-tubes in duplicates. The content of each tube was evaporated to dryness before RIA procedure. A standard curve was prepared for each batch of fifteen or twenty samples.

(iv) RIA Procedure

The tubes were arranged in duplicates and labelled Blank, Zero (100%), standards and one to fifteen or twenty samples. Buffer (100ul) was added to Blank, Zero (100%) and standard tubes. This was followed by the addition of 100ul of antiserum to all tubes. Tubes were mixed thoroughly and left at $4^{\circ}C$ for 30 minutes. After the interval, 100ul of (^{3}H) androgen was added to all tubes, mixed and kept at $4^{\circ}C$ for 4 hours or overnight.

The 'bound' was separated from the "free" by adding 100ul of dextran-coated charcoal solution to all tubes followed by centrifugation at 2000-rpm for 10 minutes at $4^{\circ}C$. The supernatants were

decanted into counting vials containing 5mls of Scintillator solution. The radioactivity was counted on Tricarb Scintillator counter (Parkard) Model 3375.

For standard curve, the cpm was plotted as a function of unlabelled androgen (pg/tube) and the quantity of steroid in each unknown sample tube, determined by interpolation.

2.2.1(d) Validation of Assay of Androgens

(i) Specificity:

Specificity of radioimmunoassay may be defined as the extent of freedom from interferences by substances other than the one intended to be measured. In this study, specificity was influenced by the combination of solvent extraction, column chromatographic separation and the specificity of the antiserum for each androgen. The specificity of androgens assayed in this study has been further improved by the inclusion of column chromatographic procedure. The efficiency of this procedure was occasionally tested by running a t.l.c. of column chromatography eluates before RIA. However, the specificity of each antiserum has been provided by the manufacturer (Table 2.1).

(ii) Sensitivity:

The sensitivity of the assay of androgens in this study was defined as the smallest amount of unlabelled steroid that can be distinguished from

zero value. This was expressed by the following method:

Blank values obtained when aliquots of glass distilled water (1ml and 2mls.) were extracted, and the extract carried through the entire procedure and then assayed using each antiserum (Table 2.2).

(iii) Accuracy:

Accuracy of assay was assessed by determining the recovery of known amounts of each androgen (i.e. 2ng; 5ng) added to chromatographic eluates of a plasma, and 0.2ng; 0.5ng to a tissue sample. The initial concentration of each androgen in the samples and the recovery experimentals were determined on the same occasion. The accuracy of the method expressed as percentage recovery of added androgens is presented in Table 2.3, page 58).

(iv) Precision:

The precision of a method represents the variation of a given set of estimations of the same sample from the mean of that set. It is usually expressed as percentage of the mean value,

(i.e. $\frac{SD}{\bar{X}} \times 100$ = Coefficient of variation (CV%).

SD = the standard deviation of the set of estimations.

$\bar{X}$ = the mean of the set of estimations.

The precision of the RIA method in this study

was assessed by determining the "intra-assay" and "inter-assay" variations of results of the same sample after a series of measurements. The procedure was as follows:

Plasma Androgens:

Intra-Assay - The concentration of four steroids were determined in duplicates in two portions of each of two plasma pools (i.e. 4 replicates of each pool).

Inter-Assay - The two plasma pools were assayed at different times in four batches of samples (Table 2.4(a) and (b) pages 60 and 61).

Prostatic Androgens:

Intra-Assay - Two portions (500mg each) each of two tissue samples were homogenised separately, extracted and chromatographed. The concentrations of androgens were determined in duplicates in each portion (i.e. 4 replicates of each sample).

Inter-Assay - The same samples were assayed at different times in four batches of samples (Table 2.4 (c) and (d) pages 62 and 63).

Specificity of Antisera for Assay of Androgens

Antisera	% Cross-reactivity with Androgens			
	T	DHT	A-DIONE	DIOL(3α 17β)
Testosterone	100	70	-	-
DHT	50	100	1	4
A-DIONE	6.8	0.76	100	< 0.001
DIOL(3α 17β)	-	-	< 0.001	100

Table 2.1

Cross-reactivity of antisera used for assay of androgens in this study are listed above. All antisera were purchased from Steranti Research Limited, London, England. The table was compiled from brochures supplied by this manufacturer.

Sensitivity of Determinations of Androgens

Androgens Assayed	Amount of Water Assayed mls	Value from Standard Curve pg/Tube	Value of Blank pg/Tube
Testosterone	1.0	<10	<10
DHT	2.0	<10	<10
A-DIONE	1.0 2.0	<10	<10
3∝ DIOL	1.0 2.0	<10	<10

Table 2.2

Sensitivity of assay of androgens was determined by taking aliquots of glass distilled water through extraction, chromatographic separation and RIA procedures. The value obtained from standard curve is compared with the Blank Value as a measure of the sensitivity of the assay.

Accuracy of Plasma Androgen Determination

Androgens	Concentration ng/ml		Recovery	
	Original	Added	ng	%
Testosterone	5.9	-		
		2.0	1.7	86
		5.0	4.5	90
DHT	1.1	-		
		2.0	1.4	70
		5.0	3.9	78
A-DIONE	1.2	-		
		2.0	1.8	90
		5.0	4.2	84
3∝ DIOL	0.4			
		2.0	1.6	80
		5.0	4.1	82

Table 2.3(a)

Recovery of steroids added to plasma extract was taken as a measure of the accuracy of the radioimmunoassay method.

Accuracy of Tissue Androgens Determination

Androgens	Concentration ng/g		Recovery
	Amount Added (ng)	Amount Measured ng/g	%
Testosterone	None	0.8	
	0.2	0.97	85
	0.5	1.22	84
DHT	None	6.4	
	2.0	8.1	85
	5.0	10.5	82
A-DIONE	None	0.1	
	0.2	0.26	80
	0.5	0.5	80
3∝DIOL	None	2.8	
	2.0	4.2	70
	5.0	7.1	86

Table 2.3(b)

Recovery of steroids added to extract of 500mg tissue homogenate as a measure of the accuracy of the RIA method.

Intra-Assay Precision of Plasma Androgens Determinations

Androgens	PLASMA POOL A			PLASMA POOL B			
	Concentration (nmol/l)		Mean ± SD	CV %	Concentration (nmol/l)		Mean ± SD	CV %
	(1)	(2)			(1)	(2)		
Testosterone	11.4	10.6	10.7±0.6	5.6	12.8	12.5	13.2±0.66	5.0
	10.8	9.9			13.4	14.0		
DHT	3.2	4.0	3.6±0.34	9.4	5.5	6.2	5.7±0.42	7.4
	3.6	3.4			5.8	5.2		
A-DIONE	5.4	4.6	4.7±0.6	12.8	3.8	4.4	4.5±0.6	13.3
	4.8	4.0			4.6	5.2		
3α DIOL	1.2	1.2	1.2±0.16	13.3	1.5	1.0	1.2±0.21	17.5
	1.0	1.4			1.2	1.2		

Table 2.4(a)

Intra-assay variations of androgens levels in two plasma samples (4 replicates of each sample). Details as in the text. Precision is expressed as coefficient of variation (CV%).

Inter-Assay Precision of Plasma Androgens Determinations

Androgens	PLASMA POOL A						PLASMA POOL B					
	BATCHES 1	2	3	4	Mean ± SD	CV %	BATCHES 1	2	3	4	Mean ± SD	CV %
Testosterone	11.4	12.2	11.8	11.1	11.6±0.5	4.3	13.0	12.5	12.8	13.6	13.0±0.6	4.6
D H T	3.4	3.6	3.0	3.8	3.5±0.3	8.6	5.6	5.5	5.2	6.0	5.6±0.33	5.9
A-DIONE	5.4	5.1	4.6	4.8	5.0±0.4	8.0	4.2	5.0	4.6	5.2	4.8±0.44	9.2
3α DIOL	1.4	1.2	1.0	1.5	1.3±0.22	16.9	1.4	1.0	1.3	1.2	1.2±0.17	14.1

Table 2.4(b)

Inter-assay variation of androgens concentrations in two plasma samples assayed in four different batches of samples. Precision of method is expressed as coefficient of variations (c.v%).

Precision of Tissue Androgens Determination (Intra-Assay)

Androgens	Tissue Sample A				Tissue Sample B			
	(1)	(2)	Mean ± SD	CV %	(1)	(2)	Mean ± SD	CV %
Testosterone	0.74 0.85	0.95 0.84	0.84±0.09	10.7	0.55 0.60	0.7 0.64	0.62±0.06	9.7
DHT	6.8 6.2	5.6 6.0	6.4±0.36	5.6	4.8 5.0	4.2 5.0	4.7±0.35	7.4
A-DIONE	0.08 0.10	0.12 0.10	0.1±0.16	16.0	0.42 0.38	0.41 0.36	0.39±0.03	7.7
3∝ DIOL	2.9 3.0	2.8 2.6	2.8±0.17	6.1	1.2 1.0	1.0 1.1	1.1±0.09	8.2

Table 2.4(c):

Intra-assay variations of androgens measured in two tissue specimens (4 replicates for each specimen). Precision is expressed as coefficient of variation (CV%).

INTER-ASSAY PRECISION OF TISSUE ANDROGENS DETERMINATIONS.

	TISSUE SAMPLE A						TISSUE SAMPLE B.					
	1	2	3	4	Mean ± SD	CV %	1	2	3	4	Mean ± SD	CV %
Testosterone	0.74	0.60	0.68	0.78	0.7 ± 0.08	11.4	0.45	0.55	0.60	0.45	0.51 ± 0.08	15.7
DHT	6.5	7.0	6.7	6.4	6.7 ± 0.26	3.9	4.6	4.4	4.8	4.4	4.6 ± 0.19	4.1
A-DIONE	0.10	0.12	0.09	0.10	0.1 ± 0.012	12.0	0.45	0.48	0.46	0.40	0.44 ± 0.03	6.8
3α DIOL	2.8	2.7	2.4	2.9	2.7 ± 0.21	7.8	1.2	1.4	1.1	1.2	1.2 ± 0.13	10.8

TABLE 2.4 (d)

Inter-assay variations of androgens in two tissue samples measured in four different batches of samples. Precision is expressed as Coefficient of Variation (CV %).

2.3. Analysis of Plasma Samples

2.3.1. Determination of Plasma Androgens:

Plasma Androgens (T, DHT, A-Dione, 3α Diol*) were determined in healthy Nigerian males and in those with BPH and CaP aged between 50 and 90 years. The groups consisted of 55 healthy individuals, 60 BPH and 12 CaP patients. The age distribution of each group is shown in Fig. 2.3.

Healthy Group: Individuals in this group were selected from patients attending Urology and General Surgery Clinics of the Lagos University Teaching Hospital for non-prostatic complaints. The average age of this group was 61.2 ± 6.9 years.

BPH Group: This group was made up of patients with confirmed prostate enlargement who were attending Urology Clinics of the Lagos University Teaching Hospital. Almost all patients in this group eventually had surgery for prostatectomy. Histology reports of the prostate tissue specimens also confirmed benign prostatic hyperplasia. The average age of these patients was 65.5 ± 8.1 years.

CaP Group: Patients in this group were diagnosed as adenocarcinoma either through prostate biospy or at surgery for prostatectomy. The ages ranged between 57 and 83 years, and averaged 67.3 ± 8.0 years.

Histology reports of tissue specimens also

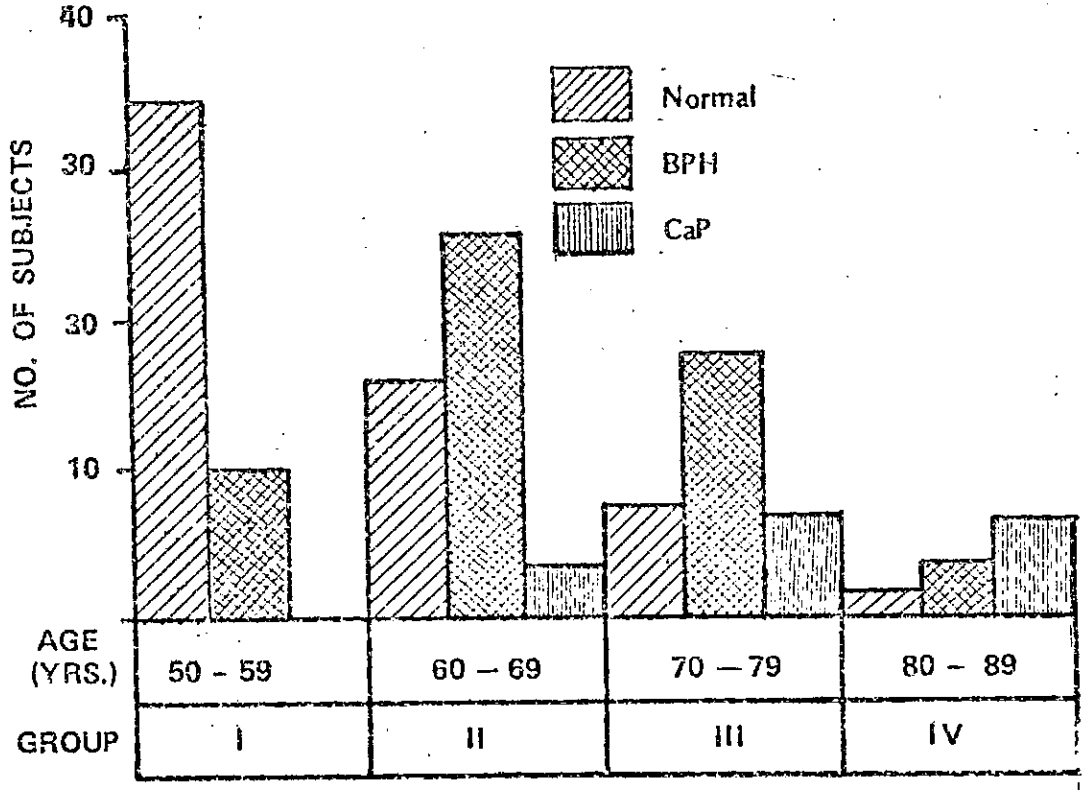


FIG. 2.3 Age distribution of Subjects included in the determinations of plasma androgens and Trace-metals

confirmed adenocarcinoma. However, no attempt was made to further classify this group into types of carcinoma or tumour grades.

(a) Preparation of Plasma Samples:

Blood samples were obtained from subjects between 8 and 10 a.m. Samples were separated and plasma obtained divided into two portions and frozen until assayed. One portion was used for androgen assay and the other for estimations of trace-metals.

(b) Preparation for Androgen Estimation:

0.5ml of each plasma sample was put into test-tubes and 100ul of each ^{3}H androgen (about 5000cpm) was then added, to monitor extraction efficiency and procedural losses of steroids. Samples were mixed by vortex and left to equilibrate at $4^{o}C$. Androgens were extracted with redistilled diethyl ether (2 x 3mls) by vortex mixing. Extracts of each sample were combined and evaporated to dryness. The residue was reconstituted in 0.2ml of Solvent-System (Heptane chloroform - methanol 85 : 15 : 5) was added to a freshly prepared Sephadex LH-20 column. Column was eluted with the solvent and 1ml. fractions of predetermined "peaks" of each androgen were collected (See Chapter 2.2.1(b)). The fractions for each androgen were combined, evaporated to dryness and reconstituted in 0.5ml of buffer solution for radioimmunoassay procedure.

(c) Determination of Recovery:

The percentage recovery of the "tracer" steroid was determined by taking 2 x 0.1ml of buffer extract (sample) and 2 x 0.1ml of a "tracer" androgen into counting vials. The vials were counted with 5ml. Scintillator solution. All counts were recorded after correcting for background readings. The percentage recovery was calculated from the equation :

$$\frac{\text{Mean cpm of sample} \times 5}{\text{Mean cpm of 100\% solution } (^3H) \text{ Androgen}} \times 100 = \text{Recovery \%}$$

Percentage recovery varied between 85% and 92.5%.

(d) Radioimmunoassay Procedure:

To assay each androgen, 2 x 0.1ml of buffer extract was taken into test-tubes (duplicates). Other procedures are detailed under radioimmunoassay procedure.

(e) Calculations:

Androgen Steroid in Sample

$$\frac{\text{Steroid/tube} \times \text{Vol. of buffer extract} \times 2}{\text{Vol. taken for Assay} \times \text{\% Recovery}} = \text{Steroid (pg/ml)}$$

Steroid (pg/ml) = Steroid (ng/l)

$$\frac{\text{Steroid (ng/l)}}{\text{Molecular Weight}} = \text{Steroid (nmol/l)}$$

2.3.2. DETERMINATION OF PLASMA ZINC AND CADMIUM

Plasma levels of zinc and cadmium were determined in the same group of subjects as for plasma androgens.

A. Plasma Zinc Levels:

Plasma zinc levels were measured in 55 Healthy Males, 60 BPH and 12 CaP patients, by Atomic Absorption Spectrophotometry (AAS) using the direct method of Smith, Batrimovitz and Pardy (1979). Plasma samples (0.5ml) were diluted initially 1:5 with glycerol:water solution (5:95 v/v). Further dilutions to obtain a total of 20mls were made when necessary. The entire diluted samples were used for analysis.

(i) Zinc Working Standards:

A series of working standards (50, 100, 150, 200 ug/dl) was prepared in glycerol:water solution (5:95 v/v) from stock standard of zinc solution (1g/dl).

(ii) Determination on AAS:

Zinc concentration of each sample was determined on Perkin-Elmer 403 Atomic Absorption Spectrophotometer at wavelength 213.8nm using Air/Acetylene gas. The baseline of the instrument was first set by aspirating glycerol:water solution (5:95 v/v) into the luminescent flame. Then working standards were sampled from the most dilute to the most concentrated, and the absorbances recorded. Samples were then aspirated and the readings recorded. Values of samples were obtained by comparison of the Absorbance of a standard with that of a sample, as stated below:

(iii) Calculation of Results:

$$\frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Conc. of Standard (ug/ml)} \times \frac{100}{0.5}$$

$$= \text{ugZn/100ml.}$$

$$\frac{\text{ugZn/100ml} \times 10}{\text{Molecular Weight}} = \text{umol/l.}$$

B. Plasma Cadmium Levels

Cadmium levels were determined in plasma samples obtained from 22 healthy males, 30 BPH and 12 CaP patients.

(i) Method of Determination

Plasma cadmium was determined by a modification of the method of Cernik and Sayers (1975) for blood cadmium. 5mls. of 3% nitric acid was added to 5mls of plasma sample and was mixed thoroughly. This mixture was then centrifuged to remove all traces of protein. The clear supernatant was made up to 10mls with distilled-deionised water before analysis on Atomic Absorption Spectrophotometer (AAS).

(ii) Cadmium Working Standards

A series of working standards ranging from 10 - 100 nmol/l of cadmium was prepared from stock cadmium chloride solution (0.5mol/l).

(iii) Analysis on AAS

Supernatant solutions from each sample and the working standards were aspirated into the flame of Perkin-Elmer 403 AAS and the absorbances were recorded. The AAS was operated at 228.8nm

wavelength and Air/Acetylene flame. Values of samples were obtained by comparing the Absorbance of sample and that of a standard.

(iv) Calculation of Results

$$\frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Conc. of Standard (nmol/ml)} \times \frac{1000}{5}$$

$$= \text{nmol } Cd^{++}/l.$$

2.4. ANALYSIS OF PROSTATIC TISSUES

2.4.1. Determination of Prostatic Androgens in Tissue Homogenates:

Intracellular concentrations of androgens (T, DHT, A-DIONE and 3α DIOL) were determined in whole tissue homogenates of prostatic tissues obtained from Nigerians aged 50 - 90 years. The group was made up of 10 Normal tissues obtained at autopsies, 60 BPH and 10 CaP obtained from retropubic prostatectomy. The "Normal" group was aged 46 - 68 years (Mean 55.9±8.0 years SD) the BPH between 50 and 80 years (Mean 64.3±9.2) and CaP between 56 and 83 years (Mean 68.9±7.8). Portions of each specimen were processed for histological confirmation of the category whether Normal, BPH or CaP.

(a) Preparation of Prostatic Tissue:

Prostatic tissue specimens were placed in ice containers and conveyed to the laboratory. In the laboratory, each specimen was rinsed in ice-cold 0.9% NaCl to remove all traces of blood. Specimens were weighed and divided into portions ranging between 500 and 800mg. Portions for androgen determination were occasionally wrapped in aluminium foil papers and kept frozen at -20°C until analysed.

(b) Preparation of Tissue for Determination of Androgens in Whole Tissue:

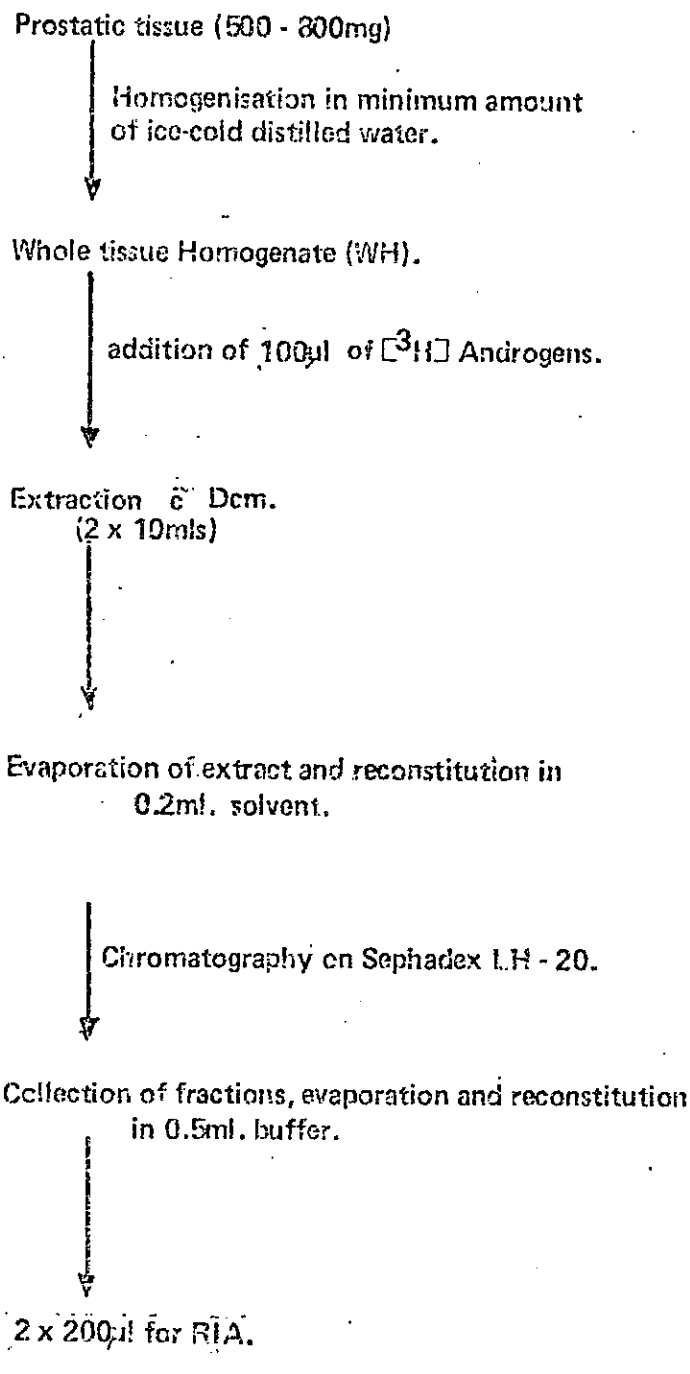
Portions of prostatic tissue specimens (500 - 800mg) were homogenised in cold distilled water. During this procedure, specimens were kept at about $4^{o}C$ or ice-cold. Each specimen required less than 10 minutes for complete homogenisation. Then 100ul of each tritiated androgen (5000cpm) was added to each homogenate as "tracer" androgen and left at $4^{o}C$ to equilibrate for 60 minutes.

(c) Extraction of Androgens from Homogenates:

Androgens in each tissue homogenate were extracted with 2 x 10mls of redistilled Dichloromethane (DCM). Extracts from each specimen were combined and evaporated to dryness and then reconstituted in 0.2ml of solvent in readiness for column chromatography (Fig. 2.4)

Fig 2.4.

FIG. 11 FLOW DIAGRAM FOR PREPARATION OF PROSTATIC TISSUE SAMPLES FOR THE DETERMINATION OF ANDROGENS.



(d) Chromatographic Separation of Androgens:

The solvent extract was added to a chromatographic column. The androgens were eluted with the solvent system (see separation of androgens) and fractions of individual androgens were collected. The fractions were evaporated to dryness and reconstituted in 0.5ml of buffer for radioimmunoassay. The percentage recovery was determined as in 2.3.1(c).

(e) Radioimmunoassay of Tissue Androgens:

For RIA, 2x0.2ml of each buffer extract was put into test-tubes. Other procedures were as stated under radioimmunoassay technique.

(f) Calculations:

Androgen steroid in tissue sample (ng/g).

Steroid/tissue sample (0.5g) =

$$(pg/\text{sample}) = \frac{\text{Steroid/tube(pg)} \times \text{Total buffer extract}}{\text{Vol. taken for RIA} \times \% \text{ Recovery}}$$

$$\text{Steroid/tissue/g (Pg/g)} = \frac{\text{Steroid/tube} \times 0.5 \times 2}{\text{Vol. taken for RIA} \times \% \text{ Recovery}}$$

$$\text{Steroid/tissue/g (ng/g)} = \frac{\text{Steroid/tube} \times 0.5 \times 2}{\text{Vol. taken for RIA} \times \% \text{ Recovery}} \times \frac{1}{1000}$$

2.4.2. Determination of Prostatic Zinc and Cadmium:

Endogenous concentrations of zinc and cadmium were determined in prostatic tissues obtained from the same group of subjects for androgen determination.

(a) Method of Determination:

Zinc and cadmium were determined by the method of Gyorkey et al (1967) which essentially was as follows: Portions of prostatic tissue specimens (500mg) were weighed and homogenised in deionised water to form 5% tissue homogenate. Equal volumes of 5% tissue homogenate and 10% Trichloracetic Acid were thoroughly mixed and then centrifuged at 2000 rpm for 20 minutes to remove proteins. The supernatant obtained was used as samples for trace-metal determinations.

(b) Determination of Zinc Concentration:

Zinc Standards: Working standards of zinc solution were prepared and analysed on AAS as previously stated (2.3.2ii).

Tissue Samples: Supernatants from tissue homogenate extraction were diluted five folds by making 4mls to 20mls with deionised-distilled water. Further suitable dilutions were made when necessary to obtain readings within the range of the standards. Samples were analysed on AAS as previously stated.

(c) Determination of Cadmium Concentration:

Cadmium Standards: Working standards of cadmium solution were prepared as previously stated and were analysed on AAS.

Tissue Samples: Aliquots of supernatant from zinc determination were analysed on AAS for cadmium concentration. Details of the instrument

were as previously stated under 2.3.2Biii.

(d) Calculation of Results.

$$\frac{A^{Test}}{A^{Std}} \times \text{Conc. of Std.(ug/ml*)} \times \frac{\text{Total Vol. of Homogenate}}{\text{Vol. of Supernatant taken}}$$

$$\times \text{Dilution factor (where applicable)} \times \frac{1}{\text{Wt. of tissue taken}}$$

$$= \text{ug } Zn^{++}/\text{g of tissue.}$$

$$\frac{\text{ug/g}}{\text{Molecular Weight } (Zn^{++})} = \text{umol/g.}$$

A^{Test} = Absorbance of Sample

A^{Std} = Absorbance of Standard

*Conc. of Standard nmol/ml. for Cd^{++}.

2.5. ANALYSIS OF SUBCELLULAR FRACTIONS

2.5.1. Determination of Subcellular Concentration of Androgens:

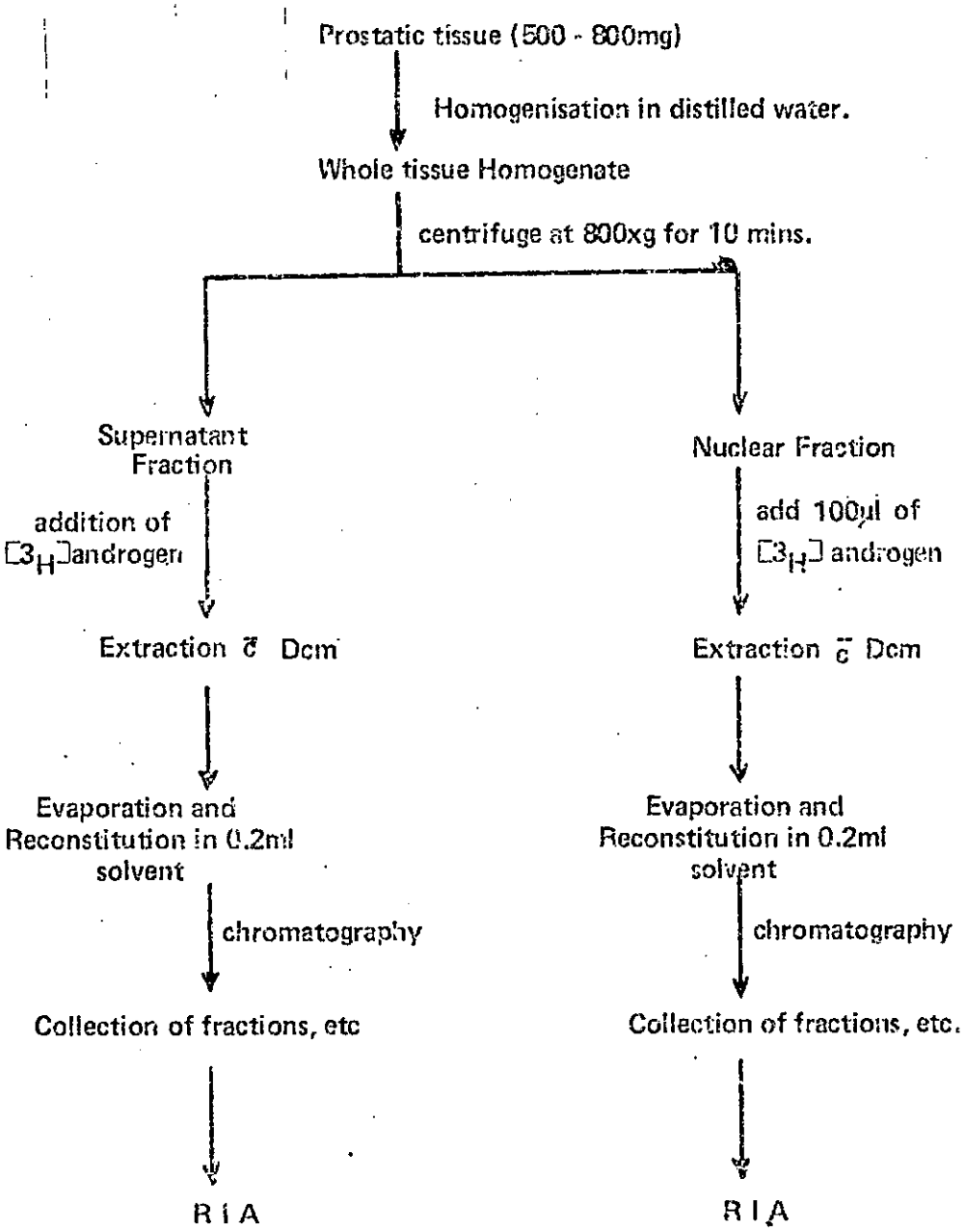
Concentrations of androgens (T, DHT, A-Dione, Diols) in the Nuclear and Supernatant fractions of prostatic tissues from Healthy males, BPH and CaP patients, were determined. This aspect of the study was performed on 10 Normal tissues (autopsy specimens) 28 BPH and 6 CaP prostatic tissues.

(a) Preparation of Subcellular Fractions: (Fig. 2.5 Page 78)

Between 500 and 800mg of each specimen was first homogenised in distilled water, and then centrifuged at 800 x g for 10 minutes in a refrigerated centrifuge. The precipitate (crude Nuclear Fraction) was resuspended twice in 10ml. Tris-Hcl buffer (p^H-7.4) containing 0.32M Sucrose, and recentrifuged on each occasion. The supernatants from the "Washings" were discarded. The Nuclear Fraction (NF) was screened for DNA to ascertain its purity and was reconstituted in 2ml of buffer preparatory for extraction and chromatography.

FIG. 2.5.

FIG. FLOW DIAGRAM FOR ISOLATION OF SUBCELLULAR FRACTIONS OF TISSUE HOMOGENATE FOR ANDROGEN DETERMINATION.



(b) Supernatant Fraction (SF)*

The Supernatant fluid from the first centrifugation was taken as a crude "Cytosol" fraction. Aliquots of this fraction were also screened for DNA contamination. Only fractions free of DNA contamination were used for subsequent analyses.

(c) Preparation of Androgen Assay:

To each fraction was added 100ul (5000cpm) of each tritiated androgen, mixed thoroughly and left at $4^{o}C$ for 60 minutes to equilibrate.

(d) Extraction, Chromatography and Assay of Androgen:

Contents of androgens of each subcellular fraction (NF and SF) were extracted with 2 x 10ml DCM. Lipid interference with extraction procedure was removed by initial addition of petroleum ether, followed by DCM extraction. The extracts from each fraction were pooled, evaporated to dryness, reconstituted in solvent system, chromatographed and assayed as previously stated.

Foot Note*

Ideally, to obtain a pure cytosol fraction, this fraction should be further centrifuged at 12,000 x g for 1h to remove mitchondria fraction, and a further 100,000 x g for 1h to remove the microsome fractions. The facilities of ultracentrifugation at these speeds were not readily available for this study

2.5.2. Determination of Subcellular Zinc and Cadmium:

Endogenous concentrations of zinc and cadmium were determined in subcellular fractions of tissues as for androgens determinations.

(a) Preparation of Tissues for Zinc and Cadmium Determinations:

Subcellular fractions of tissues were obtained as follows:

(a) Nuclear Fraction (NF):

A 5% tissue homogenate was first formed by homogenising 0.5g in 10mls of deionised-distilled water. This was then centrifuged at 800 x g for 10 minutes. The supernatant was decanted and kept as the Supernatant Fraction (SF). The precipitate was resuspended in 10mls of Tris-HCl buffer to reform a 5% homogenate. The fraction was deproteinised by adding 10ml of 10% TCA, and mixing thoroughly. The mixture was further centrifuged at 2000rpm for 20 minutes. Aliquots of the Supernatant (usually 4mls) were used for analyses after a further dilution to 20mls with deionised-distilled water.

(b) Supernatant Fraction (SF):

The first Supernatant from above procedure formed the Supernatant Fraction (SF). The total volume was determined. Equal volume of 10% TCA was added, mixed thoroughly and centrifuged as above to remove proteins.

The protein-free supernatnant was used for analysis.

(b) Determination of Zinc and Cadmium:

Zinc and Cadmium concentrations of each fraction were determined on Atomic Absorption Spectrophotometer (AAS). The procedure and instrument settings have been previously stated (see 2.3.2(A)(ii) and 2.3.2(B)(iii). Further appropriate dilutions of each sample were made to obtain readings within the range of standards.

STATISTICAL ANALYSIS OF RESULTS:

Differences between groups were evaluated statistically, using Student's 't' test for unpaired values. Differences between means were considered significant at $P < 0.05$ (5% level).

Relationship between parameters (i.e. Androgens and Trace-elements) was assessed by Pearson's correlation coefficient (r) and was considered to be significant when $p = 0.05$.

CHAPTER 3

EXPERIMENTALS

3.1 IN VITRO ANDROGEN METABOLISM BY PROSTATIC TISSUES

Prostatic tissues from 33 hyperplastic and 10 carcinomatous glands were included in this series of experiments. The BPH patients were aged between 50 and 85 years (Average - 64.3±7.8 years) while the CaP patients were aged between 56 and 83 years (68.8±7.8 years).

The experiments involved the study of *in vitro* metabolism of (a) Testosterone and (b) DHT by homogenates and subcellular fractions of tissues obtained from above patients.

3.1.1 Preparation of Tissues:

Prostatic tissues were obtained fresh at retropubic prostatectomy and conveyed to the laboratory in flasks containing ice cubes. In the laboratory, each specimen was weighed and the blood stain removed by rinsing with ice-cold 0.9% NaCl. Weighed portions (usually between 1 and 2g) were cut from the specimen and transferred to receptacles placed on ice-blocks in preparation for homogenisation.

3.1.2 Homogenisation and Preparation of Subcellular Fractions.

(a) Whole Tissue Homogenate:

One portion (1-2g) of each specimen was homogenised in a known volume of Tris-HCl buffer (p^H 7.4) such that 0.5ml. of the homogenate contained 50mg of tissue. Specimens were kept ice cold during homogenisation.

(b) Subcellular Fractions:

Another (1-2g) portion of each specimen was first homogenised as above, and then centrifuged at 800 x g for 10 minutes in a refrigerated centrifuge. The precipitate obtained formed the crude Nuclear Fraction which was reconstituted with 10mls of Tris-HCl buffer (p^H 7.4). The Supernatant formed the Supernatant Fraction.

3.1.3 Incubation Procedure

(a) Testosterone Metabolism:

0.5ml of each type of homogenate (i.e. whole homogenate, Nuclear and Supernatant Fractions) was put into each test-tube to which was added 2.0ml of buffer containing 2mM EDTA, 10% glycerol and 1mM dithrothereitol. Each tube also had 1.0ml NADPH (0.5mM). All tubes were put in a water bath at 37°C for 5 minutes to attain this temperature. Then 0.2ml of $[^3H]$ Testosterone (equivalent to 3.4×10^4 dpm) was added to each tube. Incubation was allowed to proceed at 37°C for 30 minutes in a water bath with continuous shaking mechanism.

(b) DHT Metabolism:

The same volumes of homogenates were added to another set of tubes, but with 0.2ml $[^3H]$ DHT (equivalent to 4.1×10^4 dpm) as substrate. The period and conditions of incubation were as above.

3.1.4 Termination of Incubation:

Incubation process in each tube was terminated after the chosen interval with 5mls of methanol:dichloromethane (2:1^{V}/v). Then 50u/s each of the following unlabelled steroids: Testosterone, DHT, A-Dione, 3αdiol, 3βdiol, 5αdione, was added to each tube as "carriers". The tubes were kept at 4^{o}C overnight (or until further analysis could be performed).

3.1.5 Extraction of Metabolites:

The incubation mixture in each tube was transferred to a B_{24} or B_{16} Quick fit test-tubes, where the volume was reduced. The radiometabolites were extracted with redistilled dichloromethane (2 x 10mls). Extracts of each tube were pooled, evaporated to dryness and reconstituted in 2.0ml of methanol (Methanol Extract).

3.1.6 Analysis of Products of Incubation:

The radiometabolites in the Methanol Extract were analysed by chromatographic techniques on TLC plates (Silica gel). 0.2ml aliquot of the extract was evaporated to dryness and reconstituted in a minimum amount of chloroform:methanol (1:1^{V}/v). This was put on TLC plate alongside "standard markers" and allowed to run in chloroform:ether (7:3) for about 45 minutes. At the end of the interval, the plate was removed and allowed to air dry. The spots of radiometabolites and "standard markers" were located

either by the characteristic U.V. light absorption at 240nm by 4-ene-3-oxo steroids (i.e. Testosterone and A-Dione) or by colours these metabolites produced with Iodine vapour. The spots of the "markers" and the metabolites were then marked.

3.1.7 Identification and Quantitation of Radiometabolites:

The spots of radiometabolites on the chromatogram were identified by comparing the Rf values of the "markers" with those of the metabolites. The "markers" were then aligned such that a horizontal line could be drawn between a "marker" and a metabolite. Each spot (metabolite) was then scooped into a counting vial containing 8mls of Scintillator solution. The radioactivity associated with each metabolite was then counted. Total radioactivity contained in a 0.2ml. of the original "Methanol Extract" was also determined. The amount of radiometabolites formed was expressed as a percentage of radioactivity recovered (Total radioactivity) according to the following equation:

$$\frac{[^3H] \text{ radiometabolite "X" recovered}}{[^3H] \text{ Total radioactivity recovered}} \times 100 = \% \text{ "X" formed.}$$

3.1.8 Estimation of Enzyme Activities:

The formation of metabolites was related to the activities of the enzymes as follows:

(i) Activity of 5α reductase was based on the

percentage formation of DHT and 3α(β)diols from testosterone.

(ii) Activity of 3α(β) hydrosteroid reductase was based on the percentage formation of 3α(β) diols from DHT.

(iii) Activity of 3α(β) hydroxysteroid oxidase was based on the percentage formation of DHT from 3α diol.

(iv) Activity of 17β hydroxysteroid dehydrogenase (17βOHSD) was based on the percentage formation of A-Dione and 5α Dione from testosterone.

3.2 IN VITRO EFFECT OF ZINC ON ANDROGEN METABOLISM IN PROSTATE

Varying concentrations of exogenous zinc (10^{-1}M to 10^{-12}M) were added to "incubation mixtures" in a series of experiments similar to those in 3.1. "Incubation mixtures" to which exogenous zinc solution were not added served as "Controls". The details were as follows:

3.2.1 In Vitro Effect of Zinc on Testosterone Metabolism

1ml each of zinc solution of concentration ranging from 10^{-1}M to 10^{-12}M was added to incubation mixture containing 0.5ml (50mg of tissue) of each type of homogenate and 0.2ml of (^{3}H) Testosterone. The mixture also contained 2ml of buffer (Tris-HCl p^{H} 7.4) to which 2mM EDTA, 10% glycerol had been added and 1ml 0.5mM NADPH. The period of incubation was as previously described.

3.2.2 In Vitro Effect of Zinc on DHT Metabolism:

The procedure above was repeated with (^{3}H) DHT as substrate. Other conditions were as previously stated.

3.3 IN VITRO EFFECT OF CADMIUM ON ANDROGEN METABOLISM IN THE PROSTATE:

In Vitro experiments similar to those in section 3.2 were also carried out but varying concentrations of cadmium (10^{-1} - 10^{-12}M) were substituted for zinc.

Other conditions and procedure of incubation were as described for zinc.

Controls for these experiments contained no exogenous zinc or cadmium solutions.

3.4 CALCULATION AND EXPRESSION OF RESULTS:

The effect of Zinc and Cadmium on these in vitro metabolism of androgen substrates was calculated by comparing the amount of metabolite formed from a particular substrate in the absence (Control Experiments) and in the presence (tests) of these metals.

Results are expressed as percentage increase or decrease from the maximal total activity in the "Control Experiments" (Standard Assay).

C H A P T E R 4

R E S U L T S

R E S U L T S

4.1 Relationship Between Plasma Androgens and Trace-Metals:

Plasma Testosterone (PL^{T}) was significantly lower in patients with carcinomatous prostate gland compared with age-matched BPH and Healthy Controls ($P < 0.001$). Levels of this Androgen were not significantly different in age-matched BPH and Healthy Controls, though there is overlap between the two groups (Table 4.1.1, page 91). It would appear that Plasma level of DHT (PL^{DHT}) is higher in BPH and CaP groups when compared with age-matched Healthy Control, but these differences are apparent than real as there are individual variations in levels within the two groups.

Levels of other Androgens - A-Dione and 3αdiol were not statistically different in the three groups (Table 4.1.1).

Comparison of the relative ratios of the principal androgen PL^{T} to its metabolites (Table 4.1.2) showed a ratio of 8.3 for $PL^{T}:PL^{DHT}$ in Healthy Controls and 5.8 in BPH. This suggests an apparently higher levels of PL^{DHT} in BPH subjects compared with Controls. However, the ratio of 3.9 in CaP group appears to be a reflection of low levels of PL^{T} in this group rather than high PL^{DHT} levels. Ratio of other metabolites relative to PL^{T} are shown in Table 4.1.2, page 92 .

Plasma levels of Zinc ($PL^{Zn^{++}}$) and Cadmium ($PL^{Cd^{++}}$) in the three groups of subjects are shown in

PLASMA LEVELS OF ANDROGENS IN NIGERIANS.

PLASMA ANDROGENS nmol/l	HEALTHY GROUP (n=55)	BPH (n=60)	CaP (n=12)	Significance of Means.		
Testosterone	15.5 ± 0.4 (8.2 - 22.0)	14.9 ± 0.4 (8.5 - 21.8)	10.9 ± 0.7 (7.2 - 15.2)	H V^S BPH BPH V^S CaP	p>0.10 p<.001	NS
Dihydro Testosterone	2.1 ± 0.3 (0.8 - 3.8)	2.7 ± 0.1 (1.5 - 4.6)	2.6 ± 0.2 (1.5 - 3.5)	H V^S BPH BPH V^S CaP	p>0.10 p>0.10	NS NS
Androstenedione	3.5 ± 0.5 (1.6 - 5.2)	3.4 ± 0.2 (2.0 - 5.7)	3.4 ± 0.2 (2.6 - 4.2)	H V^S BPH BPH V^S CaP	p>0.10 p>0.10	NS NS
3α Androstane-diol	1.0 ± 0.14 (0.4 - 1.8)	0.95 ± 0.1 (0.6 - 1.8)	0.95 ± 0.1 (0.4 - 1.8)	H V^S BPH BPH V^S CaP	p>0.10 p>0.10	NS NS

N.S. = Not Significant

H. = Healthy Group.

TABLE 4.1.1.

Mean ± SEM of plasma levels of androgens in Healthy, BPH and CaP subjects. The ranges of results are shown in brackets under the mean. Significance of difference of means was determined by Students 't' test for unpaired values. p is significant when $p<0.05$.

RATIOS OF PLASMA ANDROGENS AND TRACE-METALS

	Healthy Controls (n=55)	BPH (n=60)	CaP (n=12)
ANDROGENS:			
T/DHT	8.3	5.8	3.9
T/DHT + DIOL	5.5	4.3	2.8
T/A-DIONE	4.5	4.5	3.3
TRACE-METALS:			
ZINC/CADMIUM	1.0	1.2	0.5

TABLE 4.1.2.

Comparison of ratios of metabolites to the principal androgen - Testosterone, in plasma samples from the three groups of subjects. The ratio of the trace-metals in each group is also compared.

PLASMA LEVELS OF ZINC IN NIGERIANS

	Healthy Controls	BPH	CaP
Mean + SEM (Umol/l)	14.9 ± 0.5	16.5 ± 0.6	11.2 ± 0.7.
Range	8.6 - 25.2	9.8 - 30.6	7.2 - 15.0

Table 4.1. 3(a):

Levels of Zinc (Mean ± SEM umol/l) in plasma sample of 55 Healthy Controls, 60 BPH and 12 CaP Patients.

Conversion of SI Unit to conventional Units umol/l equals 6.537/ug/100ml.

PLASMA CADMIUM LEVELS IN NIGERIANS

	Health Controls	BPH	CaP
Mean ± SEM (nmol/1)	15.2 ± 0.6	15.5 ± 0.7	24.2 ± 0.9
Range	12.0 - 20.5	10.0 - 22.5	15.5 - 38.5

Table 4.1. 3(b):

Plasma levels of cadmium (Mean ± SEM nmol/1) in 22 healthy Controls, 30 BPH and 12CaP patients. Conversion of SI Unit to conventional 1nmol/1 equals 0.0 1124 ug/100ml.

Table 4.1.3(a) and (b) pages 93 and 94.

$PL^{Zn^{++}}$ is significantly higher in BPH compared with the levels in Healthy Controls ($P < 0.001$) and CaP patients ($P < 0.001$). In contrast, $PL^{Cd^{++}}$ is significantly higher in CaP patients but the levels in healthy controls and BPH are not significantly different ($P > 0.10$).

Relationship between Plasma Androgens and Trace-Metals expressed as Correlation Coefficient (r) is shown in Tables 4.1.4 and 4.1.5. There was a strong positive association between PL^{Zn} and PL^{T} in all the three groups of subjects. PL^{DHT} and $PL^{Zn^{++}}$ are also positively correlated in healthy control group ($r = 0.422$; $p < 0.001$), this relationship was not observed in other groups of patients. In healthy control groups, $PL^{A\text{-}Dione}$ was observed to be significantly related to $PL^{Zn^{++}}$; this was similar to the observation in BPH group though less significantly so.

$PL^{Cd^{++}}$ levels were not strongly correlated with Androgens in the three groups, although there were some weak associations with PL^{T} ($r = 0.501$; $p < 0.01$) and PL^{DHT} ($r = 0.346$; $p > 0.05$) in healthy controls, and PL^{DHT} ($r = 0.777$; $p < 0.01$) in CaP group (Table 4.1.5 page 97).

The ratio of PL^{Zn}:PL^{Cd} was above 1.0 in BPH group while this ratio was 0.5 in CaP group. Relationship between Zn:Cd and androgens in plasma showed a strong association between this ratio and PL^{T}, ($r = 0.465$; $p < 0.05$), PL^{DHT} ($r = 0.730$; $p < 0.001$), $PL^{A\text{-}Dione}$ ($r = 0.489$; $p < 0.05$) and $PL^{3\alpha\ diol}$

CORRELATION OF TRACE-METALS AND ANDROGENS IN PLASMA

Trace-Metal Androgens	Correlation Coefficient (r) and Significance (p)		
	Healthy Control	BPH	CaP
[µmol: nmol]			
T	0.683 p<0.001	0.525 p<0.001	0.540 p>0.05
ZINC:- DHT	0.422 p<0.001	0.079 p>0.10 NS	0.252 p>0.10 NS
A-DIONE	0.686 p<0.001	0.282 p<0.02	-0.092 p>0.10 NS
3α DIOL	-0.098 p>0.10 NS	0.038 p>0.10 NS	0.217 p>0.10 NS

TABLE 4.14.

Relationship between plasma ~~Cadmium~~ Zinc and Androgens in Healthy Controls, BPH and CaP patients, expressed as Correlation Coefficient (r). p is significant at < 0.05.

CORRELATION OF TRACE-METALS AND ANDROGENS IN PLASMA.

Trace-Metal: Androgens.	Correlation Coefficient (r) and Significance (p)		
	Healthy Controls (n=22)	BPH (n=30)	CaP (n=12)
[nmol: nmol] T.	0.501 p≥0.01	0.296 p = 0.10	0.336 p>0.10 NS
CADMIUM: DHT	0.346 p>0.05	0.169 p>0.10 NS	0.777 p<0.01
A-DIONE	0.035 p>0.10 NS	0.272 p>0.10 NS	0.319 p>0.10 NS
3αDIOL	-0.004 p≥0.10 NS	0.279 p>0.10 NS	0.338 p>0.10 NS

TABLE 4.1.5.

Relationship between plasma Cadmium and Androgens in Healthy Controls, BPH and CaP patients, expressed as Correlation Coefficient (r), p is significant at < 0.05.

ZINC: CADMIUM RATIO AND PLASMA ANDROGENS.

		[Zn: Cd] T	DHT	A-DIONE	3α DIOL.
Healthy Controls	r	0.465	0.730	0.489	0.679
	p	<0.05	<0.001	<0.05	<0.001
BPH	r	-0.049	0.214	0.058	0.115
	p	>0.10 NS	p>0.10 NS	0.10 NS	>0.10 NS
CaP	r	>0.321	0.119	-0.139	-0.104
	p	0.10 NS	>0.10 NS	0.10 NS	>0.10 NS

TABLE 4.1.6

Correlation of Zn: Cd ratio with plasma androgens expressed as Correlation Coefficient (r) Statistical significance is when $p < 0.05$.

($r = 0.679$; $p < 0.001$) in healthy controls. This association was not observed in other groups (Table 4.1.6).

4.2 Relationship Between Androgens and Trace-Metals in Prostatic Tissue Homogenates:

Prostatic tissues from CaP patients showed a significantly higher concentration of Testosterone ($P < 0.001$) and A-Dione ($P < 0.001$) than in tissues from Normals and BPH (Table 4.2.1 page 101). Prostatic DHT concentration (PR^{DHT}) was significantly higher in hyperplastic tissues than in either normal or malignant tissues ($P < 0.001$ in each case). Normal tissues contained very low testosterone but comparatively higher (PR^{Diol}) which was significantly different from BPH ($P > 0.002$) and CaP ($P < 0.001$). Table 4.2.2 page 102 shows the relative ratios of PR^{T} to other metabolites. It is observed from this Table that the sum of (PR^{DHT}) and (PR^{Diol}) relative to (PR^{T}) is much higher in Normals and BPH and lower in CaP. The high ratio of DHT to Diol in BPH is indicative of high (PR^{DHT}) in these tissues compared with normal tissues.

Endogenous concentration of Zinc (PR^{Zn}) was significantly higher in BPH compared with Normals ($P < 0.002$) or CaP ($P < 0.001$), (Table 4.2.3 page 103). Correlation of prostatic androgens with zinc shows a strong positive association between PR^{Zn} and PR^{DHT} and PR^{T} respectively in BPH ($r = 0.417$, $p < 0.001$; $r = 0.682$, $p < 0.001$), but much weaker association in both Normals ($P > 0.10$) and CaP ($P > 0.10$). There is a

weak negative correlation between (PR^{diol}) and (PR^{Zn}) in both normal and malignant tissues (Table 4.2.4, page 104). Prostatic cadmium (PR^{Cd}) is significantly higher in CaP compared with Normals or BPH ($P < 0.001$). Although there appears to be a strong positive association between ($PR^{Cd^{++}}$) and (PR^{T}) and ($PR^{A\text{-}Dione}$) respectively in normals, these associations are not statistically significant ($P > 0.10$). Similarly, the negative correlation between this trace element and (PR^{diol}) is not significant (Table 4.2.5 page 105). There is an inverse relationship between (PR^{Cd}) and DHT in Normal and PR^{T} in malignant tissues. Intraprostatic concentrations of cadmium and androgens in hyperplastic tissues are not correlated.

4.3 Subcellular Androgens and Trace-Metals:

Testosterone, DHT and A-Dione are almost evenly distributed in both nuclear and supernatant fractions of normal prostatic tissues, but 3αdiol was significantly higher in the nuclear fraction than in cytoplasmic fraction ($P < 0.001$) (Table 4.3.1, page 108). In hyperplastic tissues up to 70% of DHT measured was found in the nuclear fraction while a greater percentage (62.5%) of 3αdiol was concentrated in the cytoplasm. Other androgens were equally distributed between the two subcellular fractions of hyperplastic tissues. The cytoplasm (Supernatant Fraction) is the location of about 60% of Testosterone and A-Dione measured in malignant tissues, though the nuclear fraction also contained substantial amounts of these androgens (Fig. 4.1, page 114).

PROSTATIC ANDROGENS IN NIGERIANS.

Prostatic Androgens ng/g Wet Weight	NORMAL (n=10)	BPH (n=60)	CaP (n=10)	Significance of Means P
Testosterone	0.3 ± 0.05(SEM	0.5 ± 0.03(SEM)	7.9 ± 0.6(SE)	N V^S BPH > 0.05 BPH V^S CaP < 0.001
DHT	1.7 ± 0.18	4.9 ± 0.2	1.7 ± 0.2	N V^S BPH < 0.001 BPH V^S CaP < 0.001
A-DIONE	0.2 ± 0.03	0.2 ± 0.01	4.7 ± 0.3	N V^S BPH NS BPH V^S CaP < 0.001
3α DIOL	2.3 ± 0.24	1.2 ± 0.1(SE)	0.4 ± 0.06	N V^S BPH > 0.002 BPH V^S CaP < 0.001

NS = Not Significant.

TABLE 4.2.1

Mean ± SEM Androgens concentrations in Prostatic tissues from Normal (Autopsy Specimens) BPH and CaP. Mean between grousp are significant when $p < 0.05$.

RATIOS OF PROSTATIC ANDROGENS IN NORMAL BPH AND CaP TISSUES

	NORMAL	BPH	CaP
[ng:ng]			
T/DHT	0.2	0.08	5.4
T/DHT + DIOL	0.08	0.07	4.2
T/A-DIONE	2.7	3.3	2.0
DHT/DIOL	0.8	10.1	3.9

TABLE 4.2.2

Comparison of the concentrations of testosterone with its metabolites in the tissues from the three groups of subjects, expressed as ratios.

ZINC & CADMIUM CONCENTRATIONS IN PROSTATIC TISSUE HOMOGENATES OF NORMAL, BPH AND CaP

Trace-Metals Mean ± SEM.	NORMALS (n=10)	BPH (n=60)	CaP (n=10)
Zinc µmol/g µg/g	12.1 ± 0.8 (791 ± 55.5µg/g)	17.9 ± 0.6 (1170 ± 39.2)	2.9 ± 0.4 (189.6 ± 26.1)
Cadmium nmol/g µg/g	3.8 ± 0.63 (0.4 ± 0.07)	14.6 ± 1.1 (1.6 ± 0.12)	28.9 ± 0.37 (3.2 ± 0.04)

TABLE 4,2,3

Analysis of prostatic tissues for Zinc and Cadmium in 10 Normal 60, benign hyperplastic and 10 carcinomatous tissues.

CORRELATION OF ZINC AND PROSTATIC ANDROGENS

Trace Metals: Androgens	Correlation Co-efficient (r) and Significance					
	NORMAL (n=10)		BPH (n=60)		CaP (n=10)	
[µmol : ng] T	-0.537 p>0.10		0.682 p<0.001		0.250 p>0.10	
ZINC: DHT	0.203 p>0.10	NS	0.417 p<0.001		0.231 p>0.10	NS
A-DIONE	-0.370 p>0.10	NS	-0.115 p>0.10	NS	0.021 p>0.10	NS
3 αDIOL	-0.228 p>0.10	NS	0.057 p>0.10	NS	-0.200 p>0.10	NS

TABLE 4,2,4

Relationship between prostatic Zinc and Androgens Concentrations expressed as Correlation Co-efficient (r) Significance is at $p<0.05$

CORRELATION OF CADMIUM AND PROSTATIC ANDROGENS

Trace-Metal: Androgen	Correlation Co-efficient (r) and Significance (P)					
	NORMALS (n=10)		BPH (n=60)		CaP (n=10)	
[nmol: ng]						
T	0.458 p>0.10	NS	0.132 p>0.10	NS	-0.679 p<0.05	
CADMIUM: DHT	-0.603 p>0.05		-0.002 p>0.10	NS	-0.241 p>0.10	NS
A-DIONE	0.406 p>0.10	NS	-0.142 p>0.10	NS	-0.082 p>0.10	NS
3∝ DIOL	-0.154 p>0.10	NS	-0.204 p>0.10	NS	-0.225 p>0.10	NS

N.S. = Not Significant

TABLE 4.2.5

Relationship between prostatic cadmium and androgens concentrations expressed as Correlation Co-efficient (r). Significance is at $p<0.05$.

The nuclear fraction contained between 55 and 60% of the total zinc measured in hyperplastic tissues while about 40% was located within the cytoplasm (Fig. 4.2, page 115). Despite the overall low concentration of zinc in malignant tissue, this metal was evenly distributed between the two subcellular fractions; this was similar to the observation in normal tissues (Fig. 4.2 page 115 and Table 4.3.2, page 109).

However, a greater proportion of cadmium (55 - 70%) was measured in the cytoplasm of all tissues. Normal tissues contained very low concentration of cadmium in contrast to malignant tissues which had high levels (Fig. 4.3, page 116).

Relationship Between Androgens and Trace-Metals:

Table 4.3.3, page 110 shows that there was a strong inverse relationship between Zinc and Testosterone in both subcellular fractions of Normal tissues ($r = -0.928$ (NF); $r = -0.870$ (SF); $p < 0.001$ and $p < 0.001$ in each case). This relationship was absent in hyperplastic tissues. There were some positive associations in the supernatant fractions of malignant tissues, but these were not statistically significant. $PR^{Zn^{++}}$ was also inversely related to PR^{DHT} in the supernatant fraction of hyperplastic tissues ($r = -0.563$, $p < 0.001$), a relationship which was not observed in the nuclear fraction (Table 4.3.3). In contrast, Zinc and A-Dione were associated positively in the nuclear fraction ($r = 0.759$; $p < 0.05$)

and negatively in the supernatnant fractions ($r = -0.609$; $p < 0.001$) of BPH tissues. There were no obvious relationships between zinc and other androgens in both fractions of these group of tissues.

Cadmium concentrations were negatively correlated with Testosterone and A-Dione in the supernatant fractions of malignant tissues ($r = -0.623$, $p < 0.10$ and $r = -0.754$, $p < 0.05$), whereas this metal was only weakly related to A-Dione in the nuclear fraction of this tissue ($r = -0.668$, $p > 0.05$). Cadmium was also strongly related to 3αdiol in both subcellular fractions (Tables 4.3.5, 4.3.6). Correlations between this metal and other androgens in subcellular fractions of hyperplastic and normal tissues are shown in Tables 4.3.5 and 4.3.6 respectively.

SUBCELLULAR DISTRIBUTION OF ANDROGENS IN PROSTATIC TISSUE HOMOGENATES.

Androgens Mean ± SEM ng/g	NORMALS (n = 10)		BPH (n = 28)		CaP (n = 6)	
	NF	SF	NF	SF	NF	SF
T	0.09 ± 0.02	0.17 ± 0.03	0.14 ± 0.02 (n = 22)	0.27 ± 0.02 (n = 26)	1.7 ± 0.13	4.5 ± 0.6
DHT	0.7 ± 0.1	0.56 ± 0.09	3.5 ± 0.22	1.2 ± 0.08	0.5 ± 0.15	0.96 ± 0.18
A-DIONE	0.06 ± .007 (n=2)	0.12 ± 0.02 (n=6)	0.09 ± 0.02	0.1 ± 0.02 (n=21)	1.2 ± 0.12	2.4 ± 0.28
3α DIOL	1.06 ± 0.06	0.65 ± 0.07	0.3 ± 0.04 (n=24)	0.5 ± 0.06 (n=26)	0.12 ± 0.03	0.23 ± 0.05

TABLE 4.3.1

Concentrations of androgens in Subcellular fractions of prostatic tissues of Normal, BPH and Cap Patients. Fractions are abbreviated as NF = Nuclear Fraction; SF = Supernatant Fraction.

SUBCELLULAR DISTRIBUTION OF ZINC AND CADMIUM IN PROSTATIC TISSUES

TRACE METAL		NORMAL (n=10)	B P H (n=28)	CaP (n=6)
Zinc(µmol/g wet weight) Mean ± SE	NUCLEAR	5.7 ± 0.5	10.2 ± 0.6	1.8 ± 0.27
	SUPERNATANT	6.0 ± 0.5	7.6 ± 0.4	1.2 ± 0.17
Cadmium(nmol/g Mean ± SE	NUCLEAR	1.1 ± 0.2	4.6 ± 0.6	9.3 ± 1.7
	SUPERNATANT	2.2 ± 0.8	9.9 ± 1.2	17.4 ± 3.2

TABLE 4.3.2.

Distribution of Zinc and Cadmium in the nuclear and supernatant fractions of tissue homogenates of Normal, BPH and Cap.

CORRELATION OF CONCENTRATION OF ANDROGENS WITH TRACE-METALS IN THE NUCLEAR FRACTION

	Correlation Co-efficient (r) Significance (p)		
	NORMAL	BPH	CaP
[µmol : ng]			
ZINC: T	-0.928 p<0.001	0.050 p>0.10 NS	-0.208 p>0.10 NS
DHT	-0.276 p>0.10 NS	-0.016 p>0.10 NS	0.125 p>0.10 NS
A-DIONE	-1.000* p<0.001	0.759 p<0.001	0.495 p>0.10 NS
3∝DIOL	-0.371 p>0.10 NS	0.170 p>0.10 NS	0.166 p>0.10 NS

NS = Not Significant

TABLE 4.3.3

Relationship between prostatic Zinc and Androgens Concentrations in Nuclear Fractions of prostatic tissues from 10 Normal tissues, 28 BPH and 6 CaP. (* Only Two Samples were analysed).

CORRELATION OF TRACE-METAL AND ANDROGENS IN SUPERNATANT FRACTIONS

		Correlation Co-efficient (r) and Significance (p)					
		NORMAL		BPH		CaP	
[µmol: ng]	T	-0.870 p<0.001		-0.160 p>0.10	NS	0.611 p>0.10	NS
ZINC:-	DHT	-0.451 p>0.10	NS	-0.563 p<0.001		0.110 p>0.10	NS
	A-DIONE	0.705 p≈0.05 (n=6)		-0.609 p<0.001		0.203 p>0.10	NS
	3α DIOL	-0.435 p>0.10	NS	0.101 p>0.10	NS	0.042 p>0.10	NS

TABLE 4.3.4

Relationship between Zinc and Androgens in Supernatant Fraction of prostatic tissues.

CORRELATION OF CADMIUM AND ANDROGENS IN NUCLEAR FRACTIONS.

	Correlation Co-efficient (r) and Significance		
	NORMAL	BPH	CaP
[nmol/ng]			
T	0.578 p>0.05	0.009 p>0.10 NS	-0.206 p>0.10 NS
CADMIUM:- DHT	-0.302 p>0.10 NS	-0.134 p>0.10 NS	-0.082 p>0.10 NS
A-DIONE	-1.00 * p<0.001	0.095 p>0.10 NS	-0.668 p>0.05
3α DIOL	-0.188 p>0.10 NS	-0.198 p>0.10 NS	-0.757 p<0.05

TABLE 4.3.5

Relationship between Cadmium and Androgens in Nuclear Fraction of 10 Normal, 28 BPH and 6 CaP tissues, expressed as correlation Co-efficient (r).

(* 2 samples only).

CORRELATION OF CADMIUM AND ANDROGENS IN SUPERNATANT FRACTIONS

	Correlation Co-efficient (r) and Significance (p)		
	NORMAL	BPH	CaP
CADMIUM:- T	0.077 p>0.10 NS	- 0.249 p>0.10 NS	- 0.623 p>0.10 NS
DHT	-0.561 p<0.10 NS	- 0.285 p>0.10 NS	0.020 p>0.10 NS
A-DIONE	0.581 p<0.10 NS	0.149 p>0.10 NS	- 0.754 p<0.05
3α DIOL.	-0.333 p>0.10 NS	-0.172 p>0.10 NS	0.945 p<0.001

TABLE 4.3.6.

Relationship between Cadmium and Androgens in Supernatant Fractions of prostatic tissues.

Significance (p) is at $p<0.05$

NS = Not significant

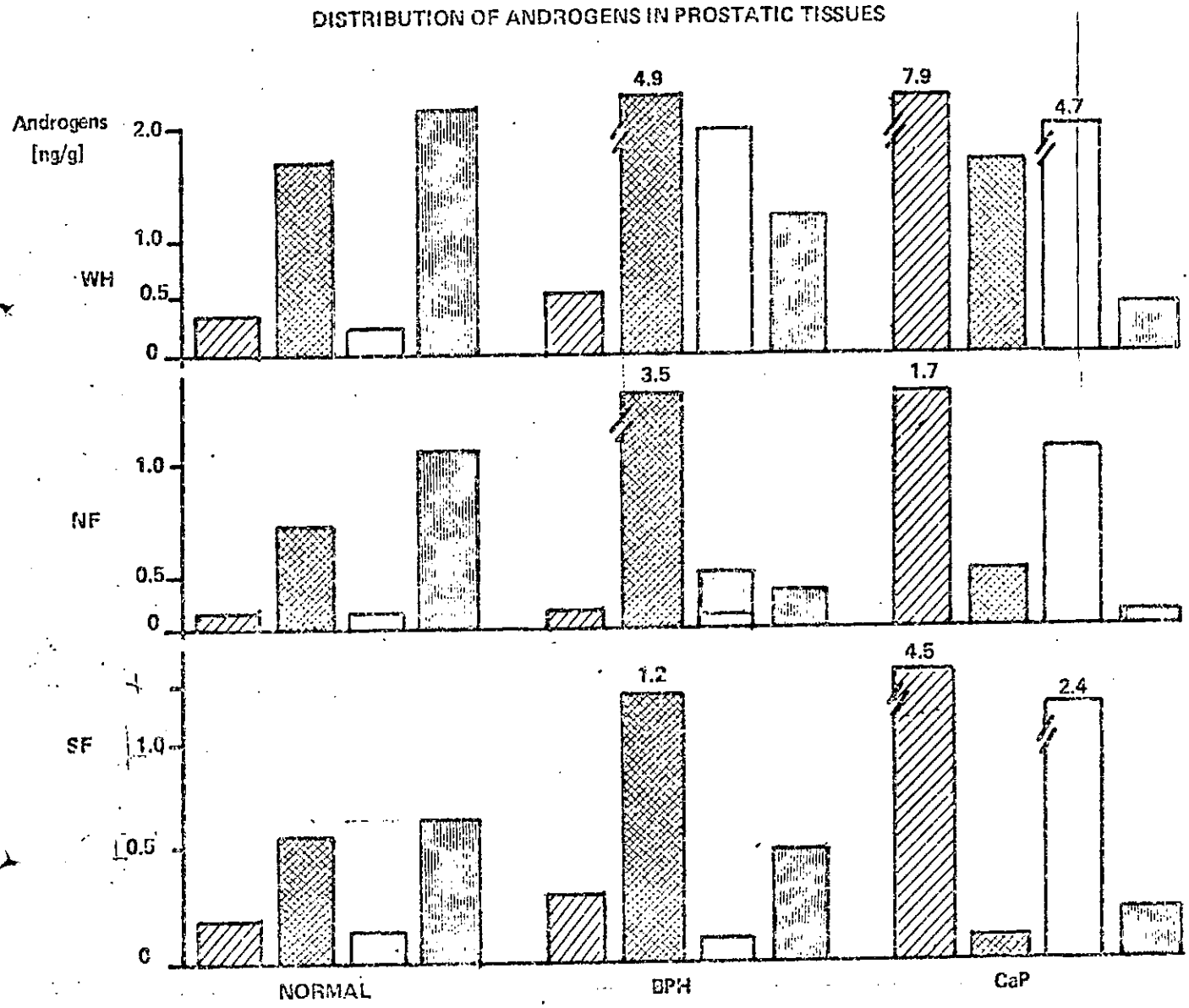


Fig. 4.1 Mean ± SEM concentration of Testosterone DHT A-DIONE and 3 α Diol in whole tissue homogenate (W.H), Nuclear Fraction (NF) and Supernatant Fraction (SF) of prostatic tissues from Normal, BPH and CaP.

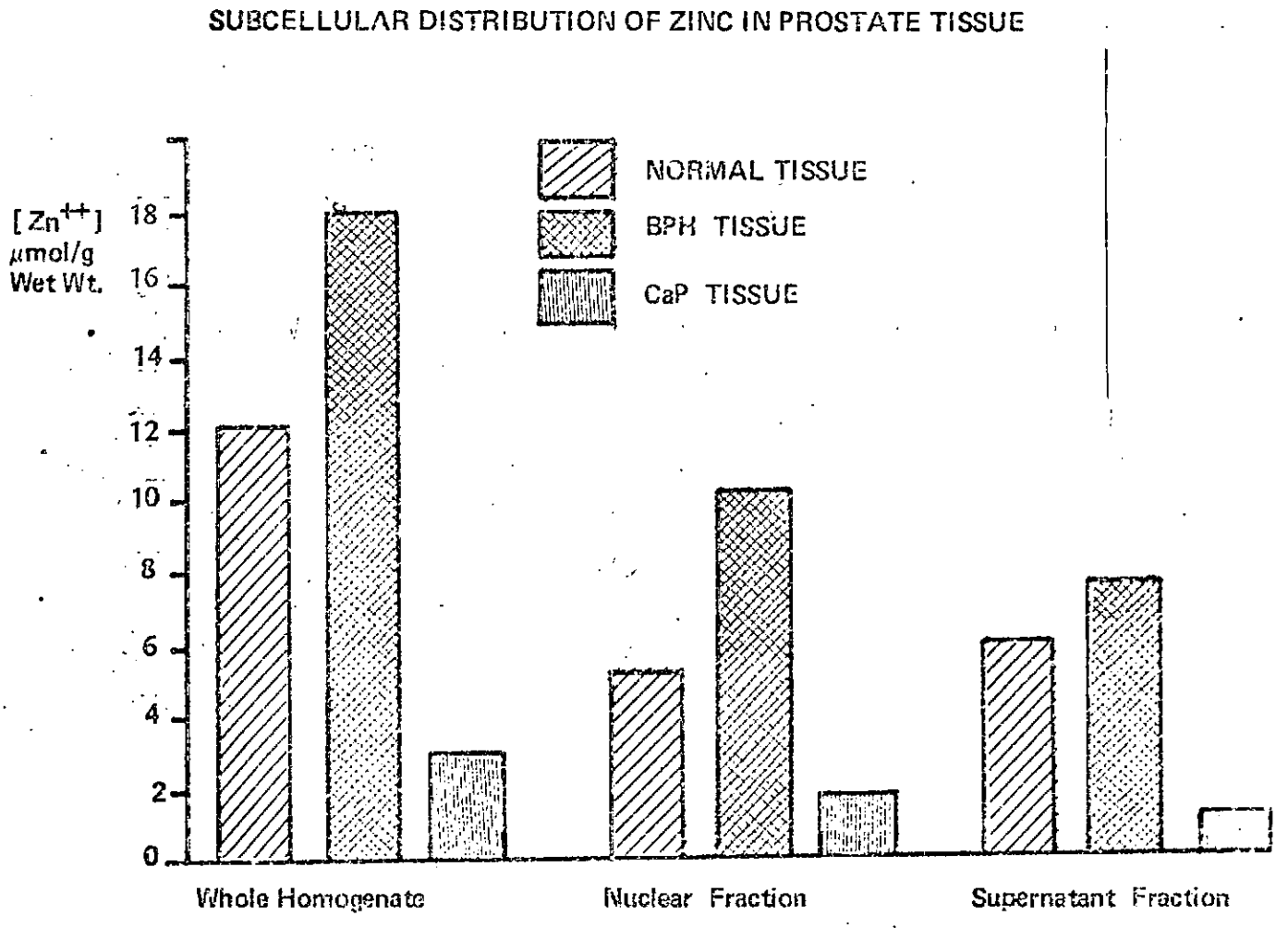


Fig. 4.2 Concentrations of zinc (µmol/g) in whole tissue homogenate, Nuclear Fraction and Supernatant fractions of Normal, BPH and CaP Tissues.

SUBCELLULAR DISTRIBUTION OF CADMIUM IN PROSTATE

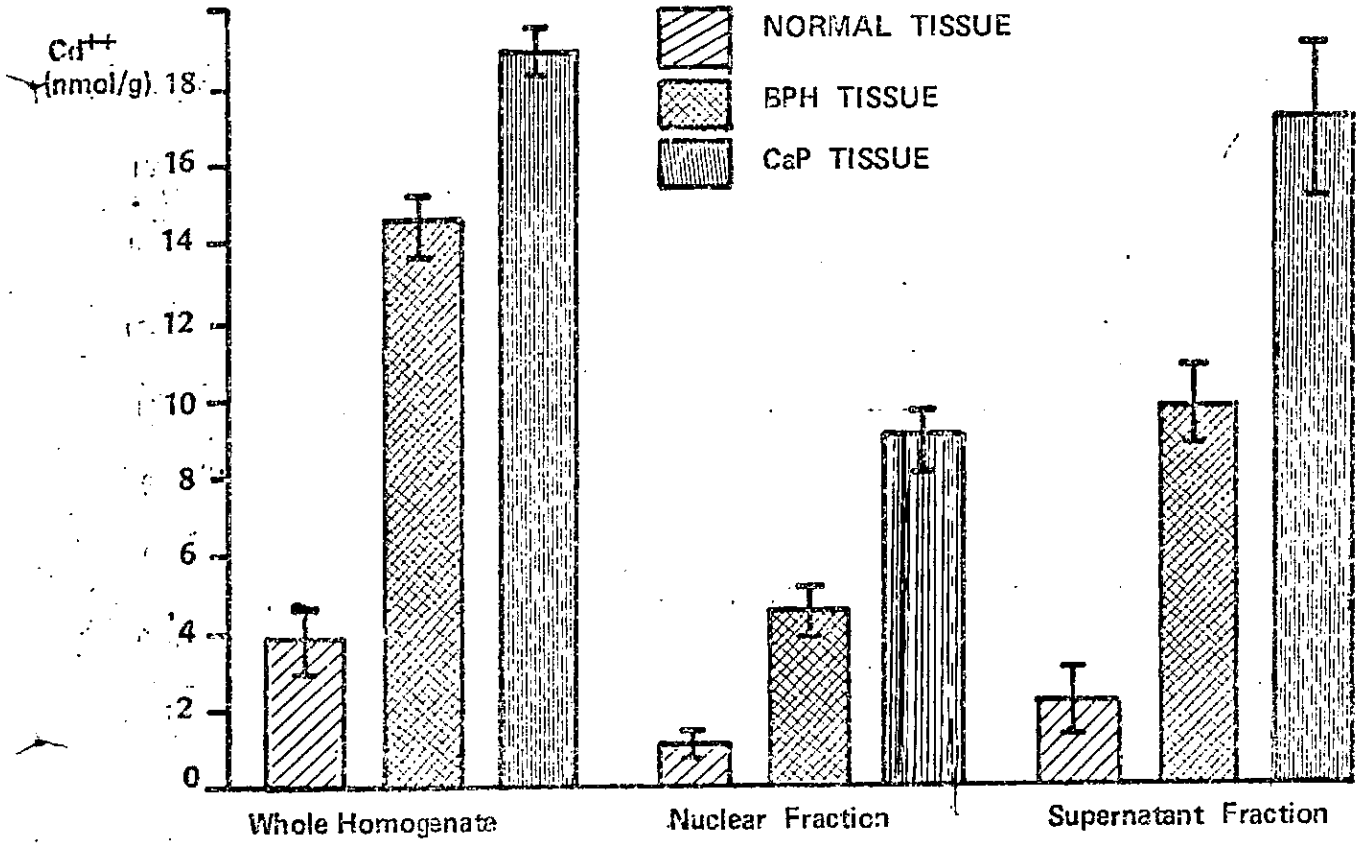


Fig. 4,3 Concentrations of Cadmium (nmol/g) in whole homogenate, Nuclear Fraction and Supernatant fractions of Normal, BPH and CaP Tissues

4.4 Androgen Metabolism in Prostate Tissues

4.4.1 Testosterone Metabolism:

In vitro incubation of testosterone produced DHT as a major metabolite in whole tissue homogenate (30.2±6.1%) in nuclear (56.3±11.2%) and in supernatant (35.1±9.8%) fractions of hyperplastic tissues. Similar results, though to a lesser degree, were obtained in malignant tissues (Table 4.4.1 page 113 and Table 4.4.2, page 119). A-Dione (23.7±7.5%) was also obtained from malignant tissues, which suggests increased 17βOHSD activity in these tissues compared with hyperplastic tissues. Supernatant fraction of malignant tissues also showed increased 17βOHSD activity (48.6±10.2%), whereas the activity of this enzyme was very low in this fraction in hyperplastic tissues. 5α reductase was the dominant enzyme in hyperplastic tissues, especially in the nuclear fraction (Table 4.4.4, page 121) though malignant tissues also showed substantial activity of this enzyme.

Hyperplastic tissues contained 3α OHSD activity which was more pronounced in the supernatant fraction compared with the low activity in the nuclear fraction. In contrast, the activity of this enzyme was very low in both nuclear and supernatant fractions of malignant tissues (Table 4.4.4, page 121).

4.4.2 Dihydrotestosterone Metabolism:

Androstenediol (3α and 3β) (42.0±13.5%) was the main metabolite obtained from in vitro conversion of

IN VITRO METABOLISM OF TESTOSTERONE IN PROSTATIC TISSUES HOMOGENATES

Androgen Metabolites	% Formation of Metabolites Mean ± SD	
	BPH	CaP
DHT	30.2 ± 6.1	21.4 ± 4.3
DIOLS	13.4 ± 3.3	7.9 ± 1.8
A-DIONE	4.5 ± 1.4	23.2 ± 7.5
5α DIONE	2.2 ± 1.1	7.5 ± 1.8

TABLE 4.4.1

Experimental formation of androgen metabolites in whole tissue homogenate of BPH and CaP. 50mg of tissue was incubated with [^{3}H] Testosterone (0.4uC) at 37^{o}C for 30 mins. Incubation medium contains 0.5mM NADPH, 2mM EDTA in a Tris-Hcl buffer (pH 7.4). Other conditions of incubation are as in the text. Results are expressed as percentage (%) of Total radioactivity recovered from incubation.

IN VITRO METABOLISM OF TESTOSTERONE IN SUBCELLULAR FRACTION OF BPH AND CaP TISSUE HOMOGENATES.

	BPH % Formation Mean ± SD		CaP % Formation Mean ± SD	
	NUCLEAR	SUPERNATANT	NUCLEAR	SUPERNATANT
DHT	56.3±11.2	35.1 ± 9.8	24.2 ± 3.7	19.9 ± 4.2
DIOLS	5.2±1.5	12.9 ± 2.3	1.0 ± 0.4	2.1 ± 0.7
Δ-DIONE	1.6%	4.9 ± 1.7	1.2	44.9 ± 9.6
5α-DIONE	<1%	1.8 ± 0.7	<1%	3.7 ± 1.7

TABLE 4.4.2

Formation of metabolites (%) in subcellular fractions of BPH and Cap tissues following incubation with [^{3}H] Testosterone. The incubation mixture contained 2mM EDTA, 0.5mM NADPH in Tris-Hcl buffer (pH 7.4). Results are expressed as % radio activity recovered from incubation.

ACTIVITIES OF ENZYMES IN PROSTATIC TISSUE HOMOGENATES

Tissue Homogenate	5 ∝ Reductase	3 ∝ (OHSD (Reductase)	17 β OHSD
BPH	45.2 ± 7.1	13.9 ± 2.9	6.7 ± 2.2
CaP	29.3 ± 5.7	7.9 ± 1.8	29.5 ± 8.0

TABLE 4.4.3

Metabolites formed from conversion of Testosterone are related to the activities of the enzymes in tissue homogenate competing for the substrate. % DHT plus % Diols = 5 ∝ reductase activity, % Diol = 3 ∝ OHSD (reductase) activity, % A-Dione plus 5-DIONE = 17 β OHSD activity.

ACTIVITIES OF ENZYMES IN SUBCELLUALR FRACTIONS OF PROSTATIC TISSUE HOMOGENATES

Tissue Homogenate	NUCLEAR FRACTION			SUPERNANTANT FRACTION		
	5α reductase	3α OHSD (Reductase)	17β OHSD	5α reductase	3α OHSD Reductase	17β OHSD
BPH	61.4 ± 12.5	5.2 ± 1.5	1.6 ± 0.6	48.0 ± 10.7	12.9 ± 2.3	6.7 ± 1.7
Cap	25.2 ± 3.7	1.0 ± 0.4	1.2 ± 0.2	21.5 ± 4.5	1.7 ± 0.9	48.6 ± 10.2

TABLE 4.4.4

Activities of enzymes in the subcellular fractions are related to the % metabolites formed from Testosterone.

Results show Mean ± SD.

IN VITRO METABOLISM OF DHT IN TISSUE HOMOGENATES

	% Formation of Metabolites Mean ± SD	
	BPH	CaP
DIOLS	43.0 ± 13.5	17.4 ± 3.9
5αDIONE	5.3 ± 2.6	33.9 12 9

TABLE 4.4.5

Metabolites (Mean ± SD) formed from incubation of [^{3}H] DHT with tissue homogenates. Results are expressed as percentage (%) of Total radioactivity recovered from incubation. The experiment was performed on 18 hyperplastic and 6 malignant tissues.

DHT in whole tissue homogenates, though the results were variable between tissues. Other detectable metabolites included 5αDione (5.3±2.6%) while a greater proportion of the substrate remained unconverted. Formation of A-Dione from DHT was very minimal and was disregarded. In malignant tissues, 5αDione was the major metabolite accounting for 33.9±12.9% while Diol formed 17.4±3.9% (Table 4.4.5, page 122).

4.5. Influence of Endogenous Concentrations of Trace-Metals on *In vitro* Metabolism of Androgens

4.5.1 Effect of Endogenous Zinc:

We examined the relationship between endogenous concentration of zinc in a tissue and *in vitro* metabolic activities of enzymes in the same tissue in order to establish if this metal influences enzyme activities in the tissue.

Results showed that there was an inverse relationship between zinc and 5α reductase enzyme activity ($r = -0.554$; $p < 0.001$); 3αOHSD ($r = -0.462$; $p < 0.001$) and 17βOHSD ($r = -0.629$; $p < 0.001$) in tissue homogenates of hyperplastic tissue (Figs. 4.4, 4.5., 4.6 pages 124 – 126)

This relationship was not observed in tissue homogenates of malignant tissues (Table 4.5.2, page 128). In subcellular fractions, the zinc content of the nuclear fraction was also inversely related to the metabolic activity of the tissue, though less significantly so (Table 4.5.1, page 127). Intraprostatic concentration of zinc in subcellular fractions of

EFFECT OF ENDOGENOUS ZINC ON 5α REDUCTASE ACTIVITY IN BPH TISSUE.

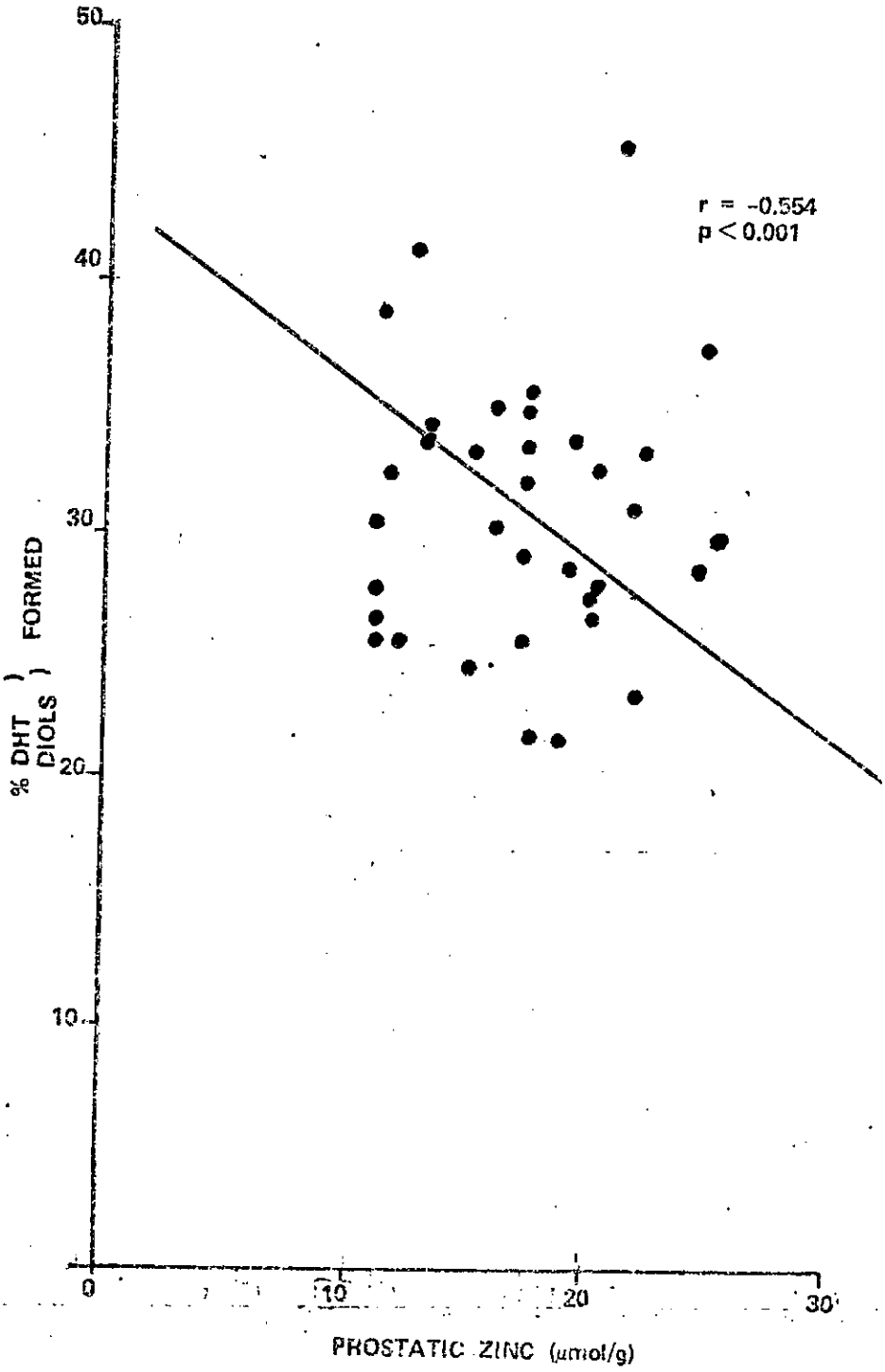


Fig.4.4 Relationship between prostatic concentration of zinc and 5α reductase activity in tissue homogenates of 33 hyperplastic tissue.

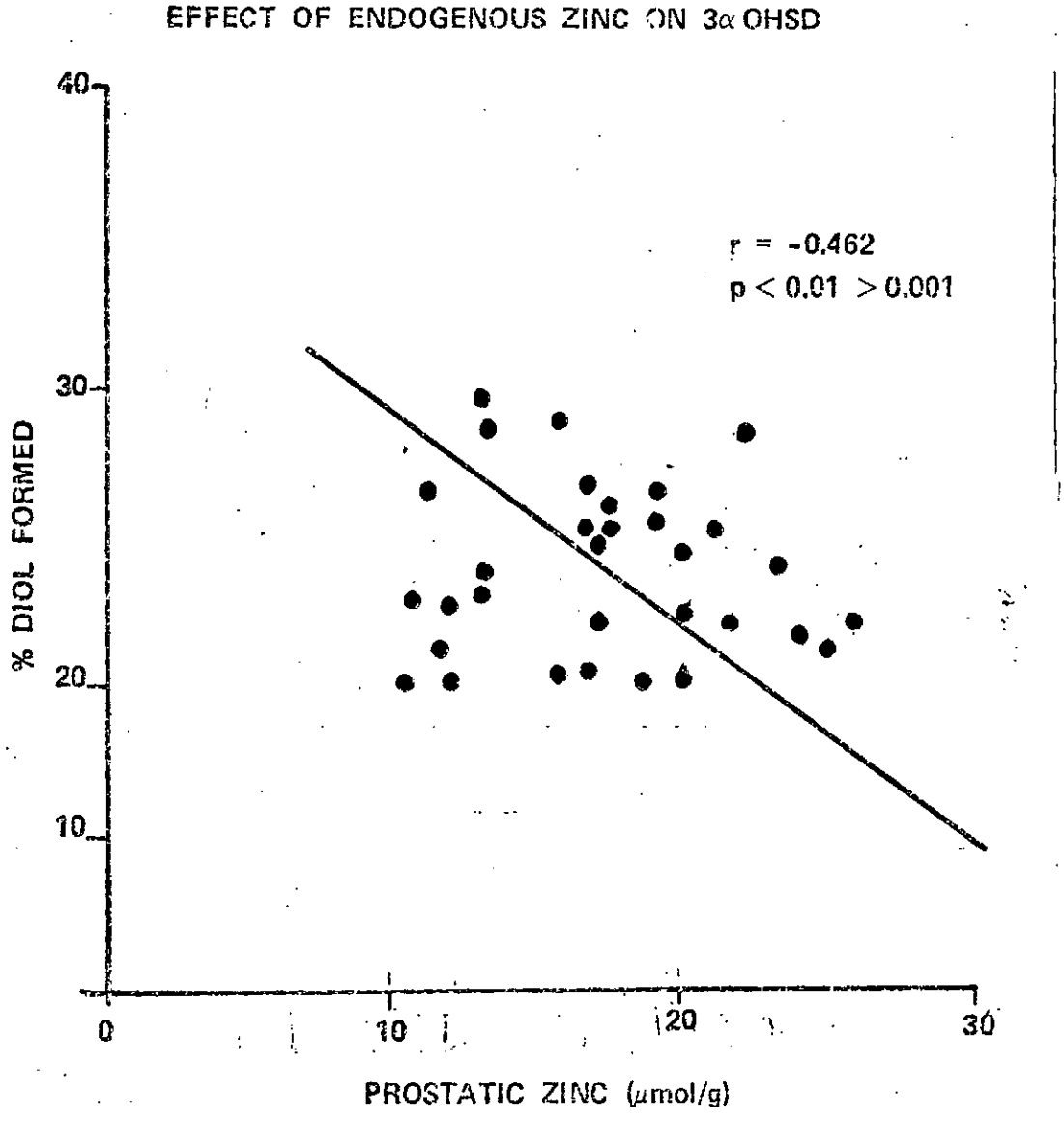


Fig. 4.5 Relationship between endogenous concentration of zinc and 3 α OHSD activity in tissue homogenates of 33 hyperplastic tissue.

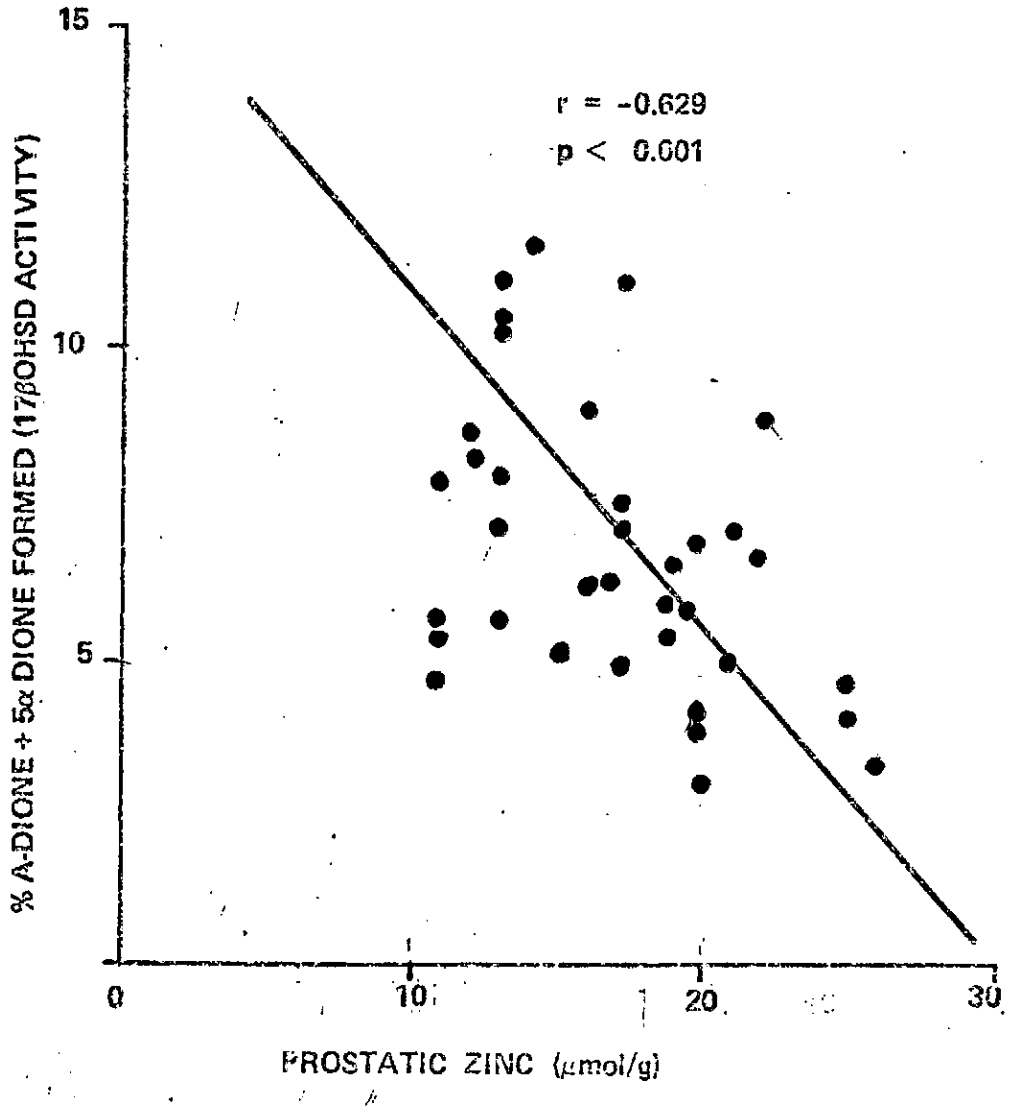


Fig. 4.6 Relationship between endogenous zinc and 17βOHSD activity in 33 hyperplastic prostatic tissues

CORRELATION OF ZINC TO ENZYME ACTIVITIES IN HYPERPLASTIC TISSUES

	WHOLE TISSUE	NF	SF
5α reductase	- 0.554 p<0.001	- 0.245 p>0.10	- 0.036 p>0.10
3α OHSD	-0.462 p<0.01>0.001	-0.655 p<0.01	- 0.217 p>0.10
17β OHSD	- 0.629 p<0.001	- 0.656 p<0.01	- 0.367 p>0.10

TABLE 4.5.1.

Relationship between endogenous concentration of Zinc and metabolic activities of 33 hyperplastic tissue homogenates, and 18 subcellular fractions. Correlation Coefficient (r) is significant at $p<0.05$.

CORRELATION OF ZINC TO ENZYME ACTIVITIES IN MALIGNANT TISSUES.

	W.H.	NF	SF
5α reductase	- 0.086 p>0.10	0.192 p>0.10	- 0.681 p<0.10>0.05
3α OHSD	- 0.071 p>0.10	0.059 p>0.10	0.439 - p>0.10
17β OHSD	0.024 p>0.10	0.612 p>0.10	- 0.052β p>0.10

TABLE 4.5.2.

Relationship between endogenous Zinc and metabolic activities of 10 malignant tissues and 6 subcellular fractions. Correlation Coefficient (r) is significant at $p<0.05$.

malignant tissue homogenates was not significantly related to any enzyme activity in the tissue (Table 4.5.2 page 128)

4.5.2 Effect of Endogenous Cadmium:

Investigations of the effect of endogenous concentration of cadmium showed no significant relationship between this metal and 5α reductase or 3αOHSD activities in hyperplastic tissues. However, a weak positive correlation was observed with 17βOHSD activity ($r = 0.347$; $p > 0.05$) (Table 4.5.3, page 130) In malignant tissues, an inverse relationship between cadmium and 5α reductase activity was observed ($r = -0.656$, $p < 0.05$), but there were no statistically significant correlation with other enzymesin this tissue (Table 4.5.3, page 130).

4.6 In Vitro Effect of Zinc:

Addition of zinc concentrations between 10^{-1} - 10^{-4} inhibited the formation of DHT from testosterone. Though the degree of inhibition varied between tissues, some tissues showed up to 60% and 50% inhibition of the standard assay in hyperplastic and malignant tissues respectively. With concentrations between 10^{-6} - 10^{-12}M, we observed an increase above standard assay (i.e. Controls) in all tissues. This stimulation was up to 95% in hyperplastic tissues and 60% in malignant tissues (Fig. 4.8(a)).

When DHT was substituted as the substrate, a similar observation was made. Concentrations of zinc between 10^{-1} and 10^{-4} inhibited the formation of

Influence of Endogenous Cadmium on Enzymes Activities

Enzyme Activities	Hyperplastic Tissues	Malignant Tissues
5αreductase	r 0.038	-0.656
	p > 0.10 NS	p<0.05
3αOHSD	r 0.0096	-0.190
	p > 0.10 NS	p>0.10 NS
17βOHSD	r 0.347	0.048
	p > 0.05	p>0.10 NS

Table 4.4.8:

Endogenous tissue concentrations of cadmium were correlated with in vitro metabolic activities of enzymes in the tissue. % formation of DHT plus Diols = 5αreductase Activity; % DIOLS = 3αOHSD Activity; % A-Dione plus 5αDione = 17βOHSD activity.

EFFECT OF ZINC ON ANDROGEN METABOLISM

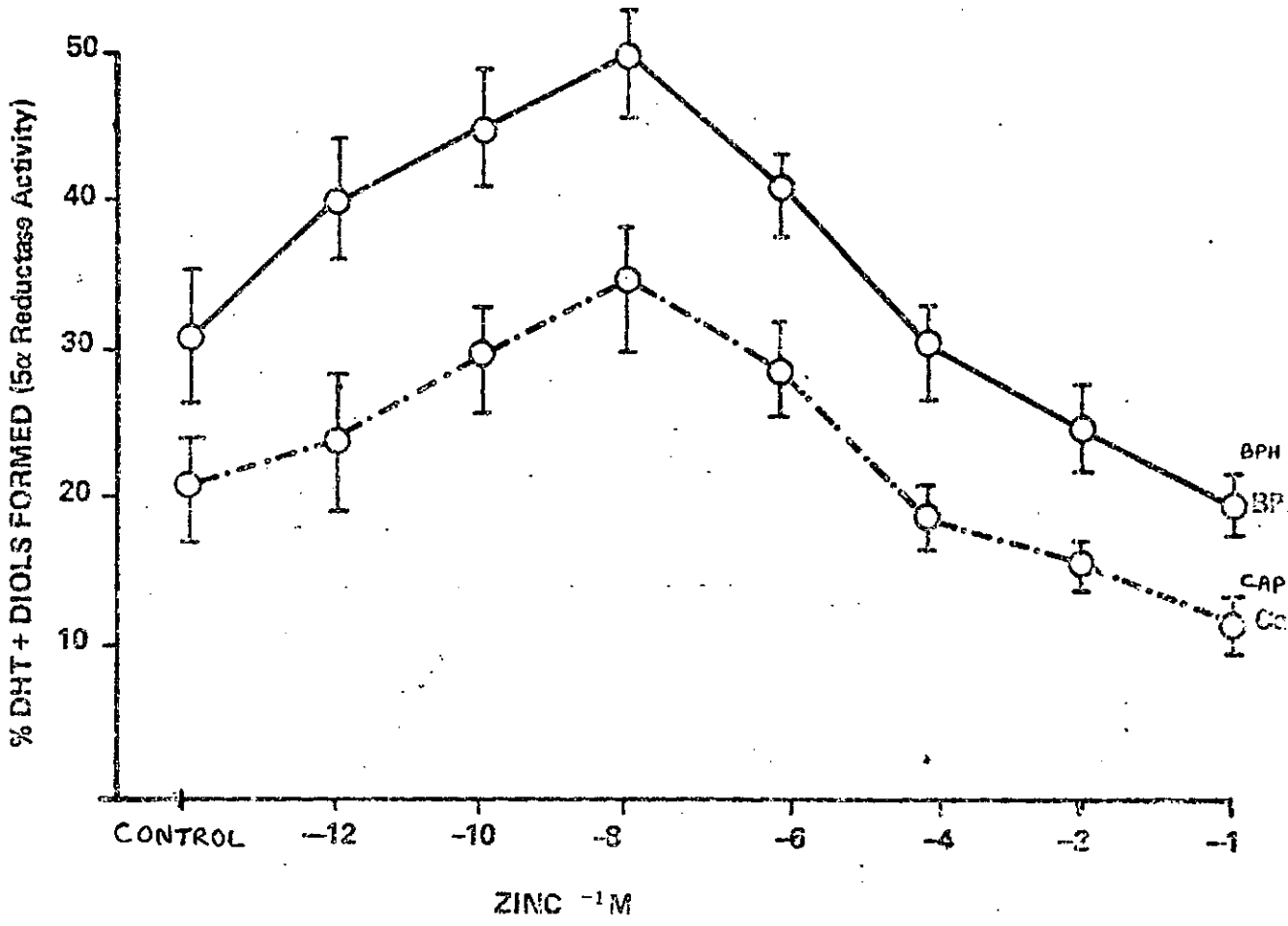


Fig. 4.8(a) In vitro effect of varying concentrations of zinc on the conversion of testosterone to DHT and DIOLS (5α reductase activity) in BPH and CaP tissues. Each point represents 26 hyperplastic and 10 malignant tissues.

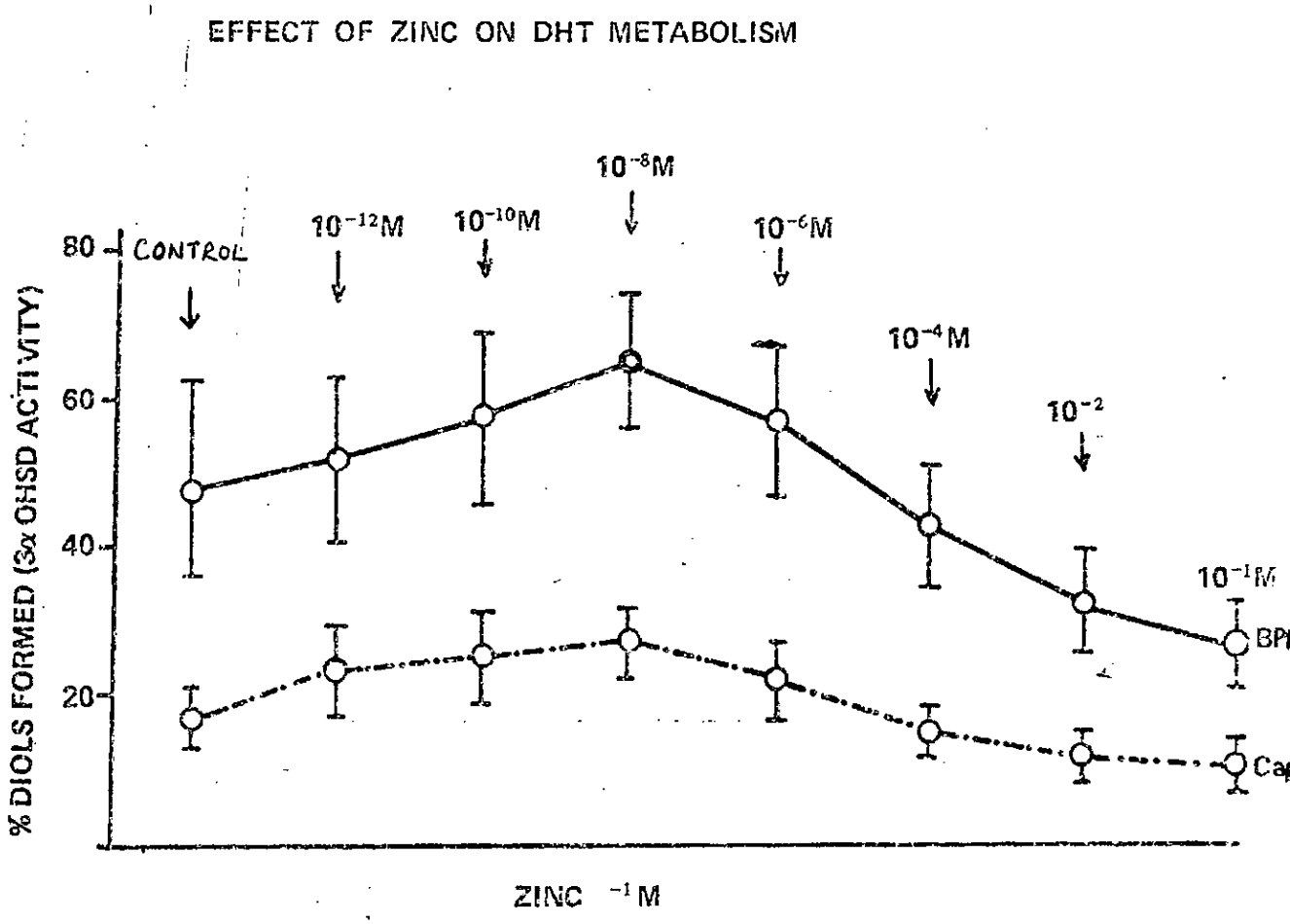


Fig. 4.8(b) In vitro effect varying concentrations of zinc on the formation of DIOLS from DHT (i.e. 3α OHSD Activity) in both hyperplastic and Malignant tissues. Each point represents the Mean ± SD of 10 hyperplastic and 6 malignant tissues.

Diol by between 40 and 60% in hyperplastic tissues while low concentrations stimulated the conversion by up to 70% of the standard assay (Fig. 4.8(b)). In malignant tissues, the stimulatory effect was 39% above the standard assay while the inhibitory effect was 44% of the standard assay.

4.7 In Vitro Effect of Cadmium:

Concentrations of cadmium above 10^{-5}M suppressed the formation of DHT and Diols from testosterone in hyperplastic tissues by between 60 and 90% of the standard assay. With concentrations of 10^{-5} - 10^{-12}M, no consistent effect was observed. Only four specimens showed increased formation of metabolites of 20-30%. In malignant tissues, the inhibitory effect with concentrations above 10^{-6}M was up to 100% in some tissues (Table 4.6.1).

With DHT as substrate, cadmium concentrations between 10^{-1} and 10^{-5}M inhibited the formation of Diols by 80-100% of the standard assay in hyperplastic tissues, while experiments with malignant tissues also showed inhibitions of up to 100% in many tissues. Additions of concentrations between 10^{-5} and 10^{-12}M had no consistent effects on the enzyme activities in these tissues (Table 4.6.2).

IN VITRO EFFECT OF CADMIUM ON ANDROGEN METABOLISM.

	BPH	CaP
	% Metabolites Formed Mean ± SD	% Metabolites Formed Mean ± SD
Control		
-1_M	57.4 ± 8.0	30.0 ± 7.9
10^{-1}	7.3 ± 1.9	1.3 ± 1.5
10^{-2}	11.8 ± 3.3	2.3 ± 0.9
10^{-4}	16.3 ± 3.4	8.5 ± 1.5
10^{-6}	30.4 ± 7.6	12.2 ± 4.6
10^{-8}	46.6 ± 12.6	16.5 ± 6.1
10^{-10}	51.7 ± 10.7	21.1 ± 4.5
10^{-12}	48.7 ± 11.2	19.4 ± 5.3

TABLE 4.6.1

Effect of additions of varying concentrations of Cd^{++} to "incubation mixture" containing (^3H) Testosterone and 18 hyperplastic and 4 carcinomatous tissues.

EFFECT OF CADMIUM ON DHT METABOLISM

	BPH	CaP
	% Metabolites Formed Mean ± SD	% Metabolites Formed Mean ± SD.
Control $_{-1m}$	40.2 ± 9.0	11.6 ± 3.3
10^{-1}	3.1 ± 2.0	0.6 ± 1.1
10^{-2}	4.9 ± 2.3	2.0 ± 1.5
10^{-4}	7.4 ± 2.4	2.6 ± 1.1
10^{-6}	14.2 ± 4.0	3.1 ± 1.2
10^{-8}	25.2 ± 6.3	4.2 ± 1.2
10^{-10}	35.6 ± 8.1	4.9 ± 1.3
10^{-12}	40.9 ± 10.4	6.1 ± 0.5

TABLE 4.6.2

In vitro effect of varying concentrations of Cadmium on DHT metabolism by 10 hyperplastic and 4 carcinomatous tissues.

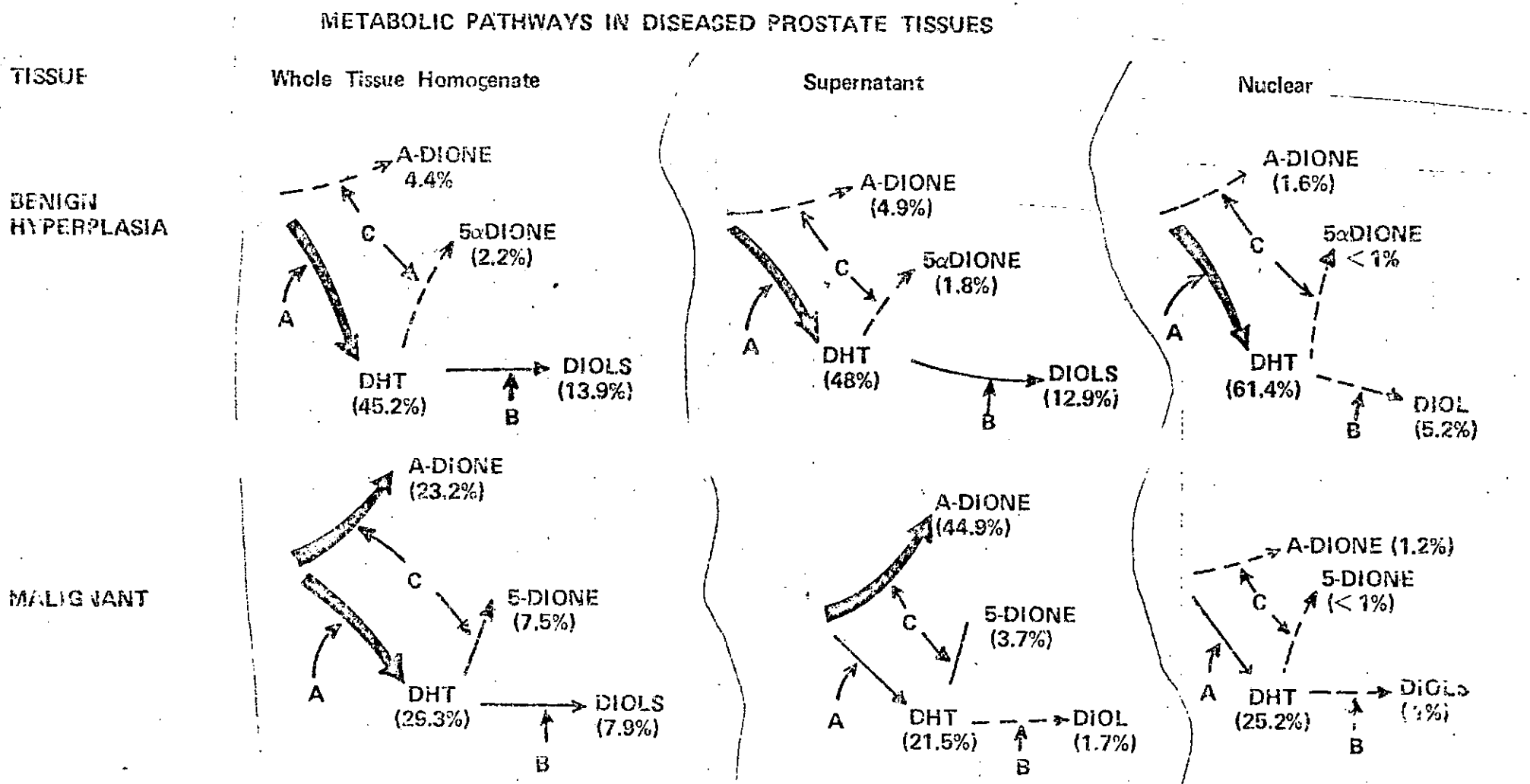


Fig. 4.7 Diagramatic demonstration of *in vitro* metabolic activities in benign hyperplastic and malignant prostatic tissues, expressed as % formation of metabolites from testosterone. Note the increase in 17βOHSD activity in malignant tissue accompanied by reduced 5α reductase activity in the subcellular fractions.

Key to Enzymes:– (A) —5α reductase; (B) — 3α OHSD; (C) —17β OHSD.

C H A P T E R 5

D I S C U S S I O N

The Unchartered sea of the
Endocrines,
Strewn as it is,
With the wrecks of Shattered
hypothesis
Where even the most wary Mariner,
May easily lose his way .....

William Boyd.

DISCUSSION

Plasma Androgens and Trace-Metals:

It is well established that the growth of prostatic tissue is androgen dependent. However, opinions differ as to the significance of higher or lower levels of circulating androgens in the development of prostatic disorders. Some workers (Hammond et al, 1978, Lukkarien 1980, Vermenlen and De Sy, 1976) contended that continuous increased androgenic stimulation enhances neoplastic development while others (Barberia et al, 1975; Ishiamamu et al, 1977; Ghanadian 1977) maintained that reduced androgenic status in the male with associated prostatic atrophy preceeds the development of carcinoma. Our findings of similar levels of testosterone in healthy controls and age matched BPH, and lower levels in CaP group, support the former view. Results of plasma testosterone levels in healthy controls, in this series, were comparable with the levels reported for Germans (Bartsch et al, 1979) and were higher than the levels in Scandinavians (Lukkarien, 1980). Furthermore, our results were significantly higher than those previously reported for Nigerians (Jackson et al, 1977; Ahluwalia et al, 1981), which suggests that the andogenic status of the Nigerian male is not significantly different from those of Caucasians or Black Americans. Therefore, the suggestions that decreased testosterone levels in Nigerians (Ahluwalia et al, 1981) or Africans (Briggs and Briggs, 1972) prevent the development of prostatic disorders in these population is not confirmed by this study. Earlier studies (Dada and Nduka, 1980; Bolarin and

Andy, 1982) have also shown that androgenic status in healthy Nigerian males of younger age is not different from that of Caucasians.

It should be noted, however, that circulating levels of testosterone are influenced by both episodic and diurnal variations, and variations in periodic fluctuations of up to 21% have been reported (Doering et al, 1975). These variations may have rendered plasma testosterone determination of limited diagnostic value and a poor discriminator of prostatic diseases. Levels of other metabolites (DHT, A-Dione and 3αdiol) were not significantly different in the three groups, though there appears to be an apparent elevated levels of DHT in BPH group.

Results of plasma zinc levels in this series are comparable to those reported for Caucasians (Willden and Robinson, 1975; Habib et al, 1980), but we recorded elevated levels of zinc in benigh hyperplastic group compared with age-matched healthy controls and CaP group. Other reports have also associated high plasma zinc level with benign hyperplasia (Jaffa et al, 1980). Our results showed decreased zinc levels in our cancer group. Similarly, decreased levels of zinc have been reported in other malignant diseases (Addink, 1960) and in epilepsy (Olatunbosun et al, 1978). Furthermore, there was a strong association between zinc and plasma testosterone in the three groups of subjects. In view of the interrelationship between testosterone and other metabolites in blood (Fig. 1.3), we examined the relationship of zinc with other metabolites. Plasma zinc is strongly associated with DHT and A-Dione in healthy controls, whereas such relationship was not

observed in diseased states. We, therefore, inferred that some level of zinc is required for the peripheral conversion of testosterone to other metabolites in healthy males, and the absence of similar observation in patients, may be due to other factors. In BPH, for example, only 50% of the DHT is derived from testosterone while 25% is contributed by A-Dione (Mahoundean et al, 1971). Besides, secretions of DHT from BPH adenoma have been suggested (Mahoundean et al, 1974). These contributions from other sources may have distorted any influence of zinc on the formation of this metabolite.

There are no previous reports of plasma cadmium determinations in prostate disorders in Nigerians. We found increased plasma cadmium levels in our cancer group compared to the healthy controls and BPH group, but these levels are still much lower than the normal range for whole blood reported for subjects in highly industrialised countries (Cernick-Sayer, 1975). Cadmium is increasingly being discharged into the atmosphere through industrial activities and cigarette smoking; a situation which is now increasingly obtainable in Nigeria.

Though increased cadmium levels have been linked with prostate cancer (Kipling and Waterhouse, 1967), available evidence is still not strong enough to suggest a causal relationship. Increased cadmium levels have been reported in cigarette smokers compared with non-smokers (Phleban and Pearson, 1979). Thind and Fischer (1976) have also recorded high plasma cadmium levels in hypertension. These reports further confirm that high blood cadmium levels may not be directly related to any malignancy. Our observation of some association between cadmium and testosterone in healthy

controls, gave rise to the examination of the role of Zn:Cd ratio in the maintenance of the androgen status of the Nigerian male. The finding of a strong association between Zn:Cd ratio and androgens in healthy controls, provided some evidence of the influence of the relative concentrations of these metals on adrogen levels in prostatic disorders. Since Zn:Cd ratio averaged 1.0 in healthy controls, it is tempting to speculate that changes in the ratio, as was found in BPH and CaP (1.2 and 0.5 respectively) may promote changes in androgenic status in diseased states. Though a causal relationship between circulating androgens and prostate pathology have not been established, it is believed that these androgens form the main source of intracellular androgens. Therefore, intracellular concentrations of androgens may provide a better reflection of the physiological state of the gland.

Intracellular Androgens and Trace-Metals:

Investigations of intracellular androgens revealed that DHT is highly concentrated in hyperplastic gland whereas testosterone and A-Dione accumulate in malignant gland. Similar observations have been reported by Habib et al (1976) and Hammond (1978). However, testosterone concentration in the cancer group in our series was much higher than have been reported (Habib et al, 1976). This difference could have arisen because of the advanced stage of the disease in our series. We also observed a higher concentration of Diol in normal tissues compared with hyperplastic or malignant tissues. The ratio of testosterone to the sum of DHT and Diol concentration was much lower in normal and hyperplastic tissues compared with CaP tissues. This

finding is indicative of active 3∝OHSD activity in normal tissues which prevents the accumulation of DHT and its attendant hyperplastic development. Since accumulation of androgens in the prostate is a consequence of testosterone metabolism in the prostate, mediated through various enzymes, these results suggest different metabolic processes in hyperplastic and malignant glands. Therefore, determinations of prostatic androgens may be a useful index to distinguish benign from malignant tissues.

In addition to DHT, hyperplastic tissues also contained elevated concentration of zinc while cadmium was concentrated in malignant tissues. In contrast, zinc was depleted in malignant gland; a result which supports the contention that the low plasma zinc observed in CaP patients was a mirror of intracellular concentrations (Jaffa et al, 1980). Above results suggest a relationship between zinc and metabolic activities in the prostate gland. Our findings of a positive relationship between zinc and DHT in hyperplastic tissues would support this suggestion.

Other studies have shown that when endogenous levels of zinc fall below 3.0ug/g dry weight, there is a reduction in intracellular DHT concentrations (Habib et al, 1976). Therefore, zinc concentrations found in normal tissues in Nigerians may be protecting the prostate gland from neoplastic changes by providing DHT sufficient for normal biological activity of the gland. Furthermore, the inverse relationship observed between cadmium and DHT in normal tissue provided a supportive evidence of the deleterious effect of high concentration of cadmium may have on normal prostatic activities, by inhibiting the provision of DHT to the cells.

Available evidence now suggests that DHT accumulation in the prostatic cell is causal to the development of hyperplasia (Isaac et al, 1983). Results of high levels of zinc in hyperplastic tissues strongly support the postulate that while normal concentrations of zinc may protect against neoplastic changes, higher concentrations may enhance DHT formation. This high concentration of zinc may inhibit further conversion of DHT, thus giving rise to accumulation of DHT with resultant hyperplasia.

A greater proportion of zinc is located in the nucleus of hyperplastic gland, where supra normal levels of DHT are also found. Similar findings have been reported for DHT (Meikle et al, 1980) and recently for zinc (Leake et al, 1984). However, we did not observe any significant relationship between zinc and DHT in the nuclear fraction, whereas an inverse relationship existed in the supernatant fraction. This conflicting finding may be the consequence of the saturable retention mechanism of zinc in the nucleus (Leake et al, 1984) which could encourage zinc loss to the cytoplasm at the expense of androgens. Similarly, it is not unlikely that androgen loss may occur during the process of isolation of subcellular fractions. This may explain the conflicting results showing a positive correlation between Zn^{++} and A-Dione in the nuclear fractions of hyperplastic and malignant tissues; a correlation which was absent in supernatant fractions of malignant tissue but not in hyperplastic tissues. The significant negative correlation between zinc, DHT and A-Dione respectively suggests that this metal may have effect on the activities of 5α reductase and 17βOHSD enzymes in the supernatant

fractions of hyperplastic tissues. In view of the negative correlation between zinc and androgens in normal tissues, it appears that this metal is linked with the low concentrations of androgens in normal tissues.

Cadmium which seems to replace zinc in zinc-depleted tissues (i.e. malignant tissues), surprisingly promotes testosterone and A-Dione formation in the nuclear and supernatant fractions of these tissues, thus supporting the results of increased concentration of these androgens in malignant tissues.

Effect of Zinc/Cadmium on Androgen Metabolism:

This study confirms the well-known transformation of testosterone to predominantly DHT in hyperplastic tissues and to a lesser amount in malignant tissues. The metabolites thus formed have been related to enzyme activities in these glands (Bruchovsky and Liekovsky, 1979; Isaac et al, 1983).

Our results show that 5α reductase activity is qualitatively lower in malignant than in hyperplastic tissues but could account for an appreciable level of DHT formation in the tissues. In contrast, 17βOHSD activity is predominant in malignant tissue, giving rise to high concentrations of A-Dione.

Similarly, studies on subcellular fractions showed high 5α reductase activity in both nuclear and supernatant fractions of hyperplastic tissues. This finding is consistent with the relative distribution of DHT between these subcellular fractions, with a high proportion in the nuclear fraction. We observed that 17βOHSD which is the predominant enzyme in malignant tissues is mainly a cytoplasmic

enzyme, in contrast to the finding in hyperplastic tissues, where this enzyme has very low activity (Fig. 4.7., page 136)

DHT is metabolised in hyperplastic and malignant tissues to 3α(β) diols as a result of some 3α OHSD activity in these tissues. Though the enzyme is predominantly located in the cytoplasm, some activity has been observed in the nuclear fraction. This is at variance with the report of Hudson (1982) which did not find any nuclear 3α OHSD. However, our result is in agreement with the views of Farnsworth (1982) and Habib et al (1979) that the activity of this enzyme remains high in hyperplastic tissues.

The results of these in vitro experiments provided further scientific explanations for the differences in the concentrations of intraprostatic androgens obtained in hyperplastic and malignant tissues, especially in the subcellular fractions. We observed that the differences between these tissues are the result of different enzyme activities. Since we could not obtain fresh normal tissues for these experiments, we could not study the enzyme activities in a normal prostate. Such studies would have explained the roles of these enzymes in the development of prostate pathology.

However, in view of the relationship between prostatic androgens and trace-metals in tissues, we could speculate that these trace-metals exhibit their influence through interactions with prostatic enzymes.

Few studies have investigated the influence of endogenous levels of trace-metals on the enzyme activities in

the prostate gland. Yet, such studies are important to the understanding of intracellular androgen formation and the pathogenesis of prostatic disorders. We examined this influence by relating the tissue concentrations of zinc and cadmium to the *in vitro* metabolic activities of these tissues. We found that concentrations of zinc is inversely related to the activities of 5α reductase, 3αOHSD and 17βOHSD in whole tissue homogenates of hyperplastic gland. Similar but less significant findings were also obtained in the nuclear and supernatant fractions. Tissue concentrations of zinc are not significantly related to enzyme activities in malignant tissues. When tissue cadmium was considered, we found that this metal positively influences 17βOHSD activity in hyperplastic tissues but was inversely related to 5α reductase activity in malignant tissues.

These results strongly suggest that some concentration of zinc is required to stimulate enzymatic activities in the hyperplastic gland but with increased cadmium levels, 17βOHSD activity is enhanced. The latter situation may result in A-Dione accumulation which may give rise to neoplastic changes. In contrast, low concentrations of cadmium could also stimulate 5α reductase activity in malignant tissues, thus providing some DHT in these tissues.

Our results support the earlier report of *in vitro* experiments by Grant et al (1971) that high concentrations of zinc cause decreased DHT formation while low concentrations enhance DHT formation, especially in the nuclear fractions of hyperplastic tissues. Wallace and Grant (1975) have also shown that low concentration of cadmium may exhibit reactions similar to that of zinc *in vitro*.

However, Sinquin et al (1982) obtained inverse relationship between endogenous zinc and 3αOHSD but not with 5αreductase. Their results might have been affected by the low concentrations of zinc in the tissues and the small number of tissues in the series.

It seems to us that the interactions in the intact prostatic cell may be quite different from what obtains in isolated subcellular fractions. Besides, in vitro experiments may not exactly represent in vivo conditions. If these assumptions are valid, these would explain the positive relationship between zinc and DHT in whole tissue homogenate and the inverse relationship noticed in the nuclear fraction. Nuclear DHT is derived from probably two or three sources: namely, (1) Direct conversion from nuclear testosterone. (2) Translocation from cytoplasm and (3) Oxidative conversion of 3αdiol to DHT. Zinc, on the other hand, is distributed between the nucleus and the cytoplasm in a sort of dynamic state. The in vivo situation, so described, could explain the differences in the interrelationship observed between zinc and androgens in tissue homogenates and in subcellular fractions.

In vitro Effect of Zinc/Cadmium on 5αreductase Activity:

Attempts have been made to study this interrelationship in vitro, by adding known quantities of exogenous zinc to androgen substrates and prostatic tissues. The results confirm that low concentrations of zinc enhance DHT and 3αdiol formation from testosterone while high concentrations inhibit such transformation. Habib (1978) has previously reported that concentrations of zinc of between

10^{-5} and 10^{-8}M stimulate DHT formation while higher concentrations cause decreased DHT formation. However, we did not observe any inhibition of the standard assay by concentrations lower than 10^{-8}M of zinc, contrary to the reports of Habib (1978;). Additions of cadmium in these *in vitro* experiments, also showed similar activity, though the stimulation was lower than with zinc while cadmium caused greater inhibition especially in malignant tissues.

In vitro Effect of Zinc/Cadmium on 3αOHSD Activity:

There are very few reports of the *in vitro* effects of zinc or cadmium on 3αOHSD activity in human prostatic tissues. Our study shows that similar to 5αreductase, zinc stimulates 3αOHSD activity at low concentrations and inhibits the activity at high concentrations. Similarly, high concentrations of cadmium almost completely inhibited the conversion of DHT to Diol, but no consistent effect was observed with low concentrations of between 10^{-5} and 10^{-12}M.

When these results are related to *in vivo*, situations where intracellular concentrations of DHT is located in the nucleus of hyperplastic gland along with high levels of zinc, it could be inferred that DHT accumulation in the nucleus is a possible consequence of the inhibition of DHT ⟶ Diol rather than T ⟶ DHT.

This study has proposed a role for zinc (and in its absence, cadmium) in the pathogenesis of prostatic disorders amongst Nigerians. It is apparent that prevention of DHT accumulation would reduce prostatic hyperplasia; this could be accomplished with high intracellular zinc concentrations. Similarly, provision of adequate intracellular

concentrations of zinc could enhance the production of DHT required for normal metabolic process and prevent the diffusion of cadmium into the cell. Cadmium does not appear to have any physiological role in the cell. Its action, especially at high concentration is suggestive of a strong enzyme inhibitor.

C O N C L U S I O N S

A N D

S U M M A R Y

CONCLUSIONS AND SUMMARY

We have shown in this study that there are no major differences in the intracellular and extracellular hormonal environment of the prostate gland in Nigerians compared with other population. However, it is our view that interactions between zinc (or cadmium) and prostatic enzymes influence intraprostatic levels of androgens available to the gland for its physiological functions.

This deduction follows from our observation that Zinc: Cadmium ratio appears to promote peripheral conversion of testosterone to DHT and Diols in healthy subjects. In diseased states, this relationship was absent, probably due to an increase or decrease in the levels of these trace-metals. In addition, endogenous tissue concentrations of these trace-metals were associated with enzyme activities in prostatic tissues.

Furthermore, zinc enhanced the formation of DHT in the cytoplasm of prostate cell; this steroid is translocated to the nucleus. We also found that zinc levels in the nucleus were positively correlated with DHT. Therefore, we inferred that high nuclear zinc may be suppressing the metabolism of DHT, thereby causing accumulation of this androgen in the nucleus. An increased concentration of DHT is associated with hyperplastic tissues, especially in the nuclear fraction.

This study therefore confirms our concept of the interrelationship between androgens and trace-metals in the pathogenesis of prostatic disorders in humans.

SUMMARY:

Other highlights of this study include the following:

1. Plasma androgens levels in Nigerians are similar to the levels in caucasians, and there is a tendency to decline with age.

2. Intracellular testosterone is higher in Nigerian cancer patients than in healthy adults or in BPH group; this parameter could be a useful diagnostic tool to confirm or eliminate malignancy.

3. Though plasma zinc levels are variable between the subjects, intracellular zinc measurements could be a useful index to distinguish malignant from non-malignant glands.

4. In view of the possible role of zinc in the development of prostate pathology, administration of the metal should be considered in prostatic cancer management especially for poor-risk patients.

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