The University of Manitoba

THE EFFECTS OF AGING ON SERUM TESTOSTERONE, TESTOSTERONE-BINDING AND SERUM GONADOTROPIN LEVELS IN HUMAN MALES.

by

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A Thesis

Submitted to the Faculty of Graduate Studies

In Partial Fulfilment of The Requirements For The Degree
Of Master of Science

Department of Physiology

Winnipeg, Manitoba

Feb 1973

To Linda

ACKNOWLEDGEMENTS

I wish to extend my sincere thanks to my supervisor, Dr. C. Faiman, for his guidance and interest throughout this study and also for the many suggestions in the preparation of this manuscript. Thanks are also extended to Dr. J.S.D. Winter and Dr. J. Moorhouse for their support and critical evaulation of this manuscript. The skillful technical assistance of Mrs. R. Poturnak, Mrs. E. Dudeck, Mrs. C. Lindsay, Mr. I. Riyaz, Mrs. B. Grant and the secretarial aid of Mrs. J. McDougall are gratefully acknowledged.

This study was supported by Medical Research Council of Canada grant MA2997.

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ABSTRACT

The relationship of testicular function to aging was assessed by measuring total testosterone, free testosterone and gonadotropin levels in 146 healthy men 20-93 years of age. Total testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentrations were measured by radioimmunoassay. The free testosterone fraction was determined by a modified charcoal adsorption technique from which can be derived an index of testosterone not bound to serum proteins.

Mean total testosterone levels remained constant until the 7th decade at which time a decline in mean testosterone levels occurred. However, the majority of these elderly subjects had levels which were still within the range of the younger men. In contrast to total testosterone levels, the free testosterone fraction declined during the 5th decade and was reduced in the majority of elderly men. FSH and LH both showed a tendency to increase during the 5th decade so that by the end of the 8th decade elevated levels were seen in the majority of men.

Although FSH and LH levels were inversely correlated with total testosterone concentrations, the correlation was better when the free testosterone fraction rather than the total testosterone concentration was considered.

In conclusion, testosterone secretion by the testes as assessed by free testosterone levels, is diminished in elderly men. This is further supported by a compensatory rise in serum gonadotropin levels which is indicative of testicular dysfunction.

REVIEW OF THE LITERATURE

STEROID-PROTEIN INTERACTIONS

A. INTRODUCTION

It is now well established that steroid hormones are bound[1] to serum proteins in the circulation. Early studies had shown that steroids were more soluble in protein solutions than in protein-free aqueous media (1). This led to the postulate that serum proteins acted as a "carrier" or solubilizing agent for the relatively insoluble hormones. However, it is now apparent that at the low concentrations present in plasma, the steroid hormones are readily soluble (2,3). It is only in recent years that the real influence of steroid-binding proteins on hormone metabolism and physiological activity has been recognized.

The major breakthrough in this field occurred in 1956 with the description of corticosteroid-binding globulin (CBG), a specific inter-α-globulin found in normal human serum. This protein has since been well characterized and serves as a model for the study of other protein-steroid interactions. It has been demonstrated that the biologically effective level of cortisol in the body is not related to the total plasma concentration of the steroid, but rather to the concentration of cortisol not bound to CBG. It has also been shown that only unbound or "free" cortisol is available for catabolism (3,4). This concept has both clinical and patho-physiological implications:

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[1]Binding refers to a loose, reversible association not involving covalent or coordinate bonds. Depending on the molecules and residues involved, binding forces may involve hydrogen, hydrophobic, electrostatic bonds or a combination of these. The locale at which this interaction occurs on the protein is called a binding site (3,5).

patients on estrogen therapy and pregnant women have elevated total plasma cortisol levels yet show no stigmata of hypercorticism since plasma CBG levels are also elevated, resulting in a concomitant reduction in biologically active free cortisol to within normal levels (3). From this example, it can be seen that the binding of a steroid by a serum protein can have a profound effect on the steroid's biological role.

The binding and physiological role of a steroid is greatly dependent upon the physical characteristics of the protein to which the steroid is bound. CBG binds cortisol and other corticosteroids with a high affinity or avidity compared to other proteins (3). Affinity may be defined mathematically as an intrinsic association constant (Kassoc): For the simple, non-covalent, reversible binding reaction between protein (P) and steroid (S) where:

$$P + S \rightleftharpoons PS$$

and assuming activities of all components are equal to their molar concentrations then: $$Kassoc = \frac{(PS)}{(P)\cdot(S)} = \frac{1}{Kdissoc}$$

Kassoc rather than Kdissociation is used as a matter of convenience and convention. It is a measure of the association-dissociation of a specific protein-steroid complex and is expressed as liters/mole (3,6, 7). CBG has a Kassoc of 5-6 $x10^8$ L/M for cortisol compared to 5-7 x 10^3 L/M by albumin for cortisol (3). (i.e. the affinity of CBG is about 100,000 times greater than that of albumin for cortisol). The high Kassoc enables the steroid to be bound even when present in low concentrations (8). The capacity or total amount of cortisol which CBG binds when all binding sites are saturated is low compared to albumin

(3,4,9). However, because of its higher Kassoc, CBG is the major binder of serum cortisol at normal physiological concentrations.

CBG has a high specificity or preference for cortisol even in the presence of other steroids with similar molecular structures. Specificity, as it relates to a protein, depends upon: 1) the limited number of steroids a protein binds, and 2) the restricted concentration of that binding protein available to the steroid (8). Evidence to date suggests only one binding site on CBG for cortisol (3). Specificity ensures the binding of a particular steroid in a milieu abundant in structually similar, yet physiologically different steroids.

Since the description and characterization of CBG, other serum proteins have been isolated and shown to bind specific steroids (2,3,8-11). The present report reviews those serum proteins responsible for binding both androgens, such as testosterone and dihydrotestosterone (DHT), and estrogenic hormones such as estradiol.

When a small amount of radioactively-labelled testosterone is added to 1 ml of plasma and electrophoresis carried out, four peaks of radioactivity are noted (Fig. 1). The shoulder (I), cathodal to the origin, represents free steroid, while one peak each is associated with the β-globulin (II), inter-α-globulin (III) and the albumin fractions (IV) (62). In an analagous fashion radioactive cortisol labels four fractions (Fig. 1). In contrast to testosterone, cortisol binds maximally to fraction III, the inter-α-protein fraction containing CBG. ^{3}H-testosterone binds maximally to fraction II, corresponding to the β-globulins. Both steroids bind to a lesser degree to the albumin fraction. In a similar manner, estradiol has been shown to

bind to the β-globulin fraction and to displace testosterone from binding sites in the β-globulin fraction, probably indicating a common binding protein (12). This protein has been identified as Testosterone-estradiol Binding Globulin (TeBG) or Sex-Steroid-binding protein (SBP) (13). The isolation, characterization, and physiological role of the proteins responsible for binding testosterone and estradiol in the three protein fractions noted above will be considered in the following sections.

B. ALBUMIN

A major outgrowth of the protein-steroid solubility studies was the finding that many steroids, including androgens and estrogens, are loosely bound by plasma albumin (1,14-18). The non-specific nature of its binding has implicated albumin in all known steroid binding systems. Attempts to determine the number of binding sites on the albumin molecule for testosterone or estradiol have been hampered by the very high capacity of albumin, which necessitates the use of large concentrations of steroids relatively insoluble in aqueous media (3). Sandberg et al (3) estimate that there are multiple binding sites for corticosterone on human serum albumin, with one relatively strong site and about 20 sites of lesser affinity. No estimate of the number of sites for testosterone or other androgenic hormones is available. However, albumin has been shown to bind pharmacological quantities of testosterone so that it would not be possible to saturate all binding sites for testosterone at normal serum testosterone concentrations (3,16,19).

Albumin has a low affinity for testosterone as compared

to TeBG or CBG (16,20-23). The exact value, expressed as the intrinsic association constant depends upon the conditions under which it is determined (pH, ionic strength of the media and most importantly temperature). For example, at $25^{o}C$, albumin has a Kassoc for testosterone of 2.4×10^{4} L/M as compared with a value of $4\text{-}16 \times 10^{8}$ L/M exhibited by TeBG for testosterone as determined by equilibrium dialysis and sephadex filtration under given conditions (24) (i.e. TeBG has an avidity approximately 10,000 fold greater than that of albumin for testosterone). Table I lists the Kassoc of albumin, TeBG and CBG for testosterone.

In a protein solution to which testosterone (ligand) is added, the fraction of ligand bound to the protein is a function of two factors: 1) the Kassoc which is a measure of the attraction between protein and ligand, and 2) the relative concentrations of ligand and protein (assuming one binding site/protein molecule) (7,8,25). However, if there is a large excess of binding sites, as occurs in normal serum containing albumin, the fraction of ligand bound is dependent only on the Kassoc and concentration of protein, as the ligand will distribute itself between the protein and unbound state in a manner dependent upon the Kassoc. Conversly, if large amounts of ligand, in this case pharmacological amounts of testosterone, are added so that all binding sites are saturated, then the addition of more ligand will result in an increase in the fraction of ligand unbound as the additional ligand can only go into the unbound state. It is important to note that no difference in the Kassoc is seen during situations of increased or decreased binding, as occurs during pregnancy or endocrine

disorders, nor has a sex difference in Kassoc been noted. As there is an excess of binding sites for testosterone in normal serum, and as the Kassoc is constant, it follows that any changes or differences observed in the amount of steroid a protein binds must be due to changes in binding protein concentration.

With this in mind, the quantitative role of albumin in testosterone binding may be appreciated from Table 2 where the percentage of total testosterone bound to albumin, TeBG and CBG is expressed. Note that unbound testosterone levels are greater in men than in women, than in pregnant women, and that TeBG plays the predominant role in testosterone binding in all groups. Albumin plays a secondary role to that of TeBG even though the serum concentration of albumin is approximately 10,000-fold greater than that of TeBG (3.5 - 5.3 gm%/100 ml (26) compared to 0.25 - 0.5 mg/100 ml (see following section)). The greater binding by TeBG is due to its greater affinity (Kassoc) for testosterone.

C. TESTOSTERONE-ESTRADIOL BINDING GLOBULIN: ISOLATION AND CHARACTERIZATION

Although earlier experiments (20,27) had suggested the presence in plasma of a non-albumin testosterone-binding component, it was not until 1966 that Mercier *et al* (21), utilizing DEAE cellulose chromatography, succeeded in isolating a protein distinct from CBG and albumin which bound testosterone with high affinity. Pearlman and co-workers (23,28) subsequently confirmed the existence of this testosterone-binding protein after measuring testosterone-binding by a Sephadex G-25 dialysis technique.

Electrophoretic mobility studies utilizing immunoelectro-

phoresis (16), paper electrophoresis (25) and polyacrylamide gel electrophoresis (PAGE) (14) have shown this protein to migrate with the β-globulins (Fraction II Fig. 1). The same conclusion was obtained by molecular sieving techniques (16).

There is good evidence that the globulin that binds testosterone also binds estradiol. This may be assumed from the following observations: 1) The mobility of the binding-proteins is the same on paper electrophoresis (18) and PAGE (14). 2) During the course of multiple chromatographic procedures on DEAE cellulose, hydroxylapatite and Sephadex, the two types of binding activity are inseparable. Also the loss of binding activity during separation is the same for both testosterone and estradiol (18). 3) The binding activity of both steroids is reduced in a similar manner by heat inactivation and is increased in pregnancy and following exogenously administered estrogens (18). 4) Both steroids compete for the same site in displacement studies although the beta-globulin has a greater Kassoc for testosterone (14,16,18,29-31).

Displacement studies have shown TeBG to bind steroids other than testosterone, but usually with much less affinity (Table 3). However, some androgens, DHT and androstanediol for example, bind to TeBG with greater affinity than does testosterone. These steroids are both significant androgens and may, in fact, be the biologically active forms of testosterone (16). In spite of this, testosterone has been shown to be the major naturally occurring circulating steroid which binds to TeBG (16).

Like many other serum proteins TeBG appears to be a

glycoprotein. It has been shown that enzymatic removal of sialic acid residues raises the isoelectric point of TeBG, an event known to occur when sialic acid residues are removed from glycoproteins (32). Although enzymatic removal alters the iso-electric point, it does not appear to affect steroid-binding activity.

Corvol et al (14), utilizing PAGE, estimate TeBG to have a molecule weight of 98,000 in plasma which is in general agreement with the findings by Gueriguian and Pearlman (28) who quote a value of 98,000-115,000 as determined by gel filtration. Similarly Rosner et al (31) find a value of 115,000 based on the mobility of this protein upon Sephadex G-200 column chromatography. These estimates differ markedly from those obtained with semi-purified preparations. TeBG isolated by alcohol preciptitation and column chromatography yields a molecule weight of 52,000 by sedementation equilibrium (13), in agreement with a value of 50,000 for TeBG purified by affinity chromatography and fractionated by PAGE. Rosner et al (31) demonstrated that partially purified TeBG could aggregate to form a larger species when all endogenous steroid was removed, an event not seen by Corvol et al (14) when 99% of ligand was removed from plasma by charcoal adsorption. The purified 50,000 molecular weight species was also shown to bind testosterone (13). The above findings suggest disaggregation of a TeBG dimer to a monomeric form during fractionation, or detachment of TeBG from a carrier molecule. The dependence of the molecular size of TeBG on the various methods of isolation remains to be investigated.

Complete purification of TeBG has not yet been reported. The major problems in purification are the loss of binding activity during isolation and the small amount of TeBG initially present in

plasma. It is possible to estimate the maximum concentration of TeBG in plasma from available data: if each molecule has only one binding site for testosterone, then it follows that the concentration of TeBG is equal to the binding capacity (M/L) times the molecular weight of TeBG. Data from Rosner (18) and Vermeulen and Verdonck (24) indicate a concentration of 5×10^{8} L/M (binding capacity x 100,000 (MW) = 5.0 mg/L for normal female serum based on a molecular weight of 100,000 or 2.5 mg/L based on a molecular weight of 50,000.

As with CBG, evidence to date suggests there is only one binding site on TeBG (18) and that under given conditions the intrinsic association constant of TeBG for testosterone is the same regardless of sex or endocrine status (33). Therefore any difference in binding which occurs between different sexes or differing endocrine conditions (such as pregnancy or thyrotoxicosis) must be due to changes in serum concentrations of TeBG. This is analagous to CBG and thyroxine-binding globulin (TBG) which have been shown to increase or decrease in concentration in response to differing endocrine environments (pregnancy, cirrhosis of the liver etc.) (3,4,11). Table 4 lists the binding capacity of TeBG for testosterone. There is a significant difference in binding capacity between men, women and pregnant women (14,24,34-36) (pregnant>women>women>men). Certain endocrine disorders such as hyperthyroidism can also affect binding capacity (34).

Because of its greater specificity and binding affinity as compared to albumin, TeBG plays the predominent role in testosterone binding in blood (Table 2). TeBG binds approximately 96%, 83% and 60% of total blood testosterone in pregnant women, women and adult

males respectively.

D. CORTICOSTEROID-BINDING GLOBULIN

CBG is known to bind many steroids including testosterone, but with a lower affinity than for cortisol (8,25,37,38). It is presently felt that CBG does not play a significant role in binding testosterone at physiological levels (23,24,33) even though it has a higher association constant for testosterone than does albumin (Table 1). The lower binding of CBG is probably due to the lower serum concentration of CBG relative to albumin (2.6 - 4.3 mg/100 ml (4) compared to 3.5 - 5.3 gm/100 ml (26)) and the fact that the majority of binding sites on CBG are occupied by corticosteroids at physiological concentrations (24,33). Vermeulen et al (24,33) estimate CBG to bind less than 3% of the total testosterone concentration (Table 2).

E. ROLE OF PROTEIN-STEROID INTERACTION IN PLASMA

The possible role or significance of steroid "carriers" is still under dispute. Most theories are based on studies with CBG. However, these can be extended to include TeBG.

The most popular role assigned to the steroid carriers is that of a buffer mechanism which protects the tissues against abrupt changes in hormone secretion and utilization rates (39). Steroid is transferred to the target tissue as a result of competition for steroid between circulating protein and cellular binding sites. Most investigators feel that it is the free fraction which is regulated in this manner (2). As previously stated, if there are excess serum binding sites for a particular steroid, the fraction of "free" steroid will remain

relatively constant so that changes in utilization or secretion rates will result in much smaller changes in the free or bound steroid fraction than would occur if protein were absent. For example, about 20% of serum cortisol, which is essential to normal hepatic function, is removed from the blood in its passage through the liver. The concentration gradient of unbound cortisol from the periphery to the center of the liver lobule is relatively small under physiological conditions. When the liver is perfused by protein-free cortisol solutions, the gradient is much higher. In the latter instance cortisol is almost completely removed from the circulation by the liver in a single passage (39). Thus, the presence of plasma binding ensures that the most distant parts of the liver are exposed to necessary amounts of cortisol. The buffer concept has several shortcomings however. Some species, the rhesus monkey for example, have a capacity for corticoids well below normal plasma levels so that increased corticoid secretion will directly increase unbound levels of corticoids (2), a finding not in keeping with the above concept.

If, as most authors feel, the free steroid fraction is important, the concentration of biologically active steroid may be much lower at the cellular level than indicated by total serum steroid concentrations. In this case binding proteins would ensure transport of necessary amounts of hormones to distant target tissues without exposing intervening tissues to pharmacological concentrations of biologically active "free" steroid.

Other authors feel that it is the protein-bound fraction which may be important. CBG-bound cortisol has been shown to penetrate liver cells and to be degraded there (2). Some cells may only accept

bound steroid (transfer by carrier), a theory which may explain why there are two thyroxine carriers (TBG and Thyroxine-binding prealbumin): If two classes of cells exist, as occurs for cathecholamines (2), each class may specifically recognize only one of the two protein carriers.

Another role for binding proteins may be that of a storage mechanism in blood which prevents steroids from being adsorbed to the walls of blood vessels, protects steroids against chemical attack in the blood, prevents undue losses through the kidney and/or prevents catabolism by the liver.

It is of interest to note that there are individuals who, for genetic reasons, lack thyroxine-binding globulin, the major binding protein for serum thyroxine. Since these individuals are eumetabolic (40), it seems difficult to assign a physiologic role to this binding protein. It is obvious therefore that more work needs to be done in order to determine the physiologic significance of binding proteins.

F. THE EFFECTS OF PROTEIN BINDING ON THE BIOLOGICAL ACTIVITY AND METABOLISM OF TESTOSTERONE

It has been demonstrated that CBG-bound cortisol is biologically inactive (41,42). As the Kassoc of TeBG for testosterone is of the same magnitude as that of CBG for cortisol, it may be expected that TeBG bound testosterone is not biologically active. There is, in fact, evidence to support this concept: 1) Total testosterone, is considered an indicator of androgenicity. However, in pregnancy (33,43-46) and in hyperthyroidism (33,47,48) for example, moderate increases in plasma testosterone levels are seen without the usual stigmata of virilization. This apparent discrepancy

can be explained in terms of testosterone-binding. Free testosterone is actually lower in pregnancy than in the non-pregnant state (0.2 - 0.27% vs. 0.9 - 0.96%) (Table 2). The decreased free testosterone is the result of increased binding activity in the blood (16,17,23-25, 30,34,44,49,50). Similarly, free testosterone levels are found to be in the normal range in hyperthyroidism (17,33,34,48,51).

2) Metabolism of testosterone is also affected by binding.

a) Mowszowicz et al (52) demonstrated that when testosterone was bound to a serum protein extract, its in vitro aromatization by human placental microsomes was delayed. A similar inhibitory affect of albumin was observed, but only at concentrations above the physiological range.

b) Lasnitzki and Franklin (53) reported the addition of human pregnancy serum, with a high capacity for testosterone (Table 3), to the incubation mixture inhibited the effect of testosterone on rat prostatic tissue to a higher degree than did human male serum with its lesser capacity for testosterone.

c) If testosterone catabolism were a function of the unbound testosterone concentration, then there should be a correlation between the free concentration and the metabolic clearance rate (MCR) of testosterone. Vermeulen et al (54) demonstrated a positive correlation between free testosterone levels and MCR. Low free testosterone levels, which occur during pregnancy and hyperthyroidism, are generally accompanied by a low MCR (51) whereas elevated free testosterone levels are associated with an increased MCR at normal blood production rates. Vermeulen et al (34) and Bardin et al (55) showed that the MCR of

testosterone was lower in women than men. This is understandable in that women have lower free testosterone concentrations then do men (Table 2) so that less testosterone is available for catabolism.

It appears therefore, that the free testosterone concentration is a better index of androgenicity then is the total testosterone concentration and that TeBG-bound testosterone is biologically inactive.

G. REGULATION OF TESTOSTERONE-ESTROGEN BINDING GLOBULIN LEVELS

a) Effects of Estrogen and Testosterone

The regulation of the binding globulin concentration is still speculative. Estrogen may be a factor since males receiving estrogen have elevated testosterone-binding levels (25,30,49). This is analagous to the elevation in CBG and TBG levels which also occurs following estrogen administration (4,10). Both acute and chronic administration of testosterone to normal females result in an increase in the fraction of unbound testosterone towards the level seen in males (30,34,56) suggesting that androgen levels play a part in regulating binding levels or that the ratio of testosterone to estrogen is the determinant.

b) Thyroid Status

Administration of thyroxine or triiodothyronine has been shown to increase the binding of testosterone in both men and women (51). The finding of elevated estradiol, thyroxine and triiodothyronine levels in hyperthyroid males by Chopra et al (57) may explain the increased binding observed in these individuals (34) since both estrogens and thyroid hormone are known to increase testosterone binding in sera (51,49,25,30).

TESTOSTERONE SECRETION AND BINDING WITH AGE

In cross-sectional surveys, markedly different levels of total testosterone concentration and testosterone binding are seen: at different periods of pubertal development; between the sexes; and with advancing age.

A. NEONATAL AND CHILDHOOD LEVELS

The total testosterone concentration of umbilical venous blood at term is not significantly different from that in the non-pregnant female nor does a fetal sex difference appear evident (43,44, 46). However, if the male fetus were secreting testosterone, the total testosterone level would be expected to be higher in male arterial umbilical blood then in female arterial blood. Venous umbilical blood, on the other hand, would not be expected to show this difference, as rapid and extensive aromatization of androgens occurs in the placenta, tending to minimize any sex difference. Saez and Bertrand (58) found a significant difference between umbilical arterial and venous concentrations in male but not in female fetuses whereas Forest et al (50), August et al (43) and Mizuno et al (46) did not (Table 5). Thus, no firm conclusions regarding a fetal sex difference in total testosterone concentration at term can be made at this time.

Testosterone-binding is low in cord blood at term resulting in higher levels of unbound testosterone (1.3 ng/100 ml) than found in maternal blood (0.37 ng/100 ml) or in non-pregnant females (0.44 ng/100 ml) (43,50). No fetal sex difference was noted in free testosterone levels when sampled from arterial or venous vessels (50, 59). August et al (43) explained the lack of virilization expected in

newborn females with elevated levels of unbound testosterone to a need for still higher free testosterone concentrations, the anti-androgenic effects of progesterone or both. Plasma binding levels are slightly below or within the normal female range in prepubertal boys (17,33,34,43,61) and girls (36). Total testosterone levels in prepubertal girls remain slightly below that of adult women while total testosterone concentrations of prepubertal males remain in the normal adult female range (43,60,62).

B. PUBERTY

At puberty, male testosterone levels increase until the adult male range is reached (Table 6), and sex characteristics have developed (33,43,60-66,102).

Testosterone-binding also changes at puberty in males. As total androgen levels increase, binding decreases from the adult female range to the adult male range (Table 2 and Table 6) (17,33,34, 43,61). Only slight increases in total testosterone concentrations and binding is seen in girls at puberty (36,43,60,62).

C. OLD AGE (MALES)

Histologically there are no marked changes in testicular structure in the aged testes except for some thinning of the germinal epithelium and a slight reduction in the number of Leydig cells. The tubules are of adult proportions but may be slightly distended. The interstitium is also less fibrous (67,68).

The endocrine status of the aged testes is still unsettled. There are few studies of total testosterone concentrations and

testosterone-binding in aged males and the published data are contradictory: Gandy et al (69), Coppage et al (70) and Kent and Acone (71) were unable to demonstrate a significant difference in mean total plasma testosterone concentrations between young (approximately 16-40 years) and aged men (40-80 years of age) whereas Vermeulen et al (33, 72-74) report, notwithstanding striking exceptions, decreasing plasma testosterone levels after the 6th decade when compared to levels between adolescence and age fifty. Similarly, Persky et al (75) report a lower mean testosterone value in men 31-66 years of age compared to men 17-28 years. Most investigators did report a greater range or scatter in total testosterone concentrations in aged men compared to younger men (33,70,72-74).

Urinary testosterone excretion studies have yielded a tendency towards lower testosterone secretion in males after age forty (76-80).

Testosterone-binding is increased in aged males (33,73,74) (Table 4). Vermeulen et al (72) report 2.08% of the total testosterone concentration to be unbound in males 20-50 years in contrast to 1.36% for males 70-90 years of age. Kent and Acone (71), Vermeulen et al (72) and Persky et al (75) report a reduced MCR of testosterone in male senescence, no doubt due, in part, to reduced free testosterone levels (see Effects of binding). The modest fall in total plasma testosterone levels and MCR is associated with a decrease in testosterone blood production rates (BPR) (72,75,81). Kent and Acone (71) found the reduction in BPR in elderly males to be non-significant while Persky et al (75) found the BPR to be approximately one-half that of a group of younger males. Lipsett (81) reports the BPR of old men to be

approximately two-thirds that of young men.

Thus in old age normal to low-normal total testosterone levels may be seen despite decreases in BPR. This may be the result of decreased MCR (or utilization) due to a reduced free testosterone fraction.

THE PITUITARY-GONADAL AXIS

A. FEEDBACK CONCEPTS

The testis subserves two functions, namely a germinal function (spermatogenesis) and an endocrine function, the secretion of hormones, mainly androgens. Regulation of testicular function is mediated principally by the pituitary gonadotropic hormones: Luteinizing hormone (LH) or interstitial cell stimulating hormone (ICSH) and follicle stimulating hormone (FSH). Biosynthesis of testicular androgens is regulated directly by the action of LH on the Leydig cells; however, the role of FSH on androgen secretion is less clear. There is evidence to suggest that FSH is necessary for maintenance of normal germinal epithelium (82-84) while both LH and FSH appear to be necessary for complete spermatogenesis.

It is customary to consider the hypothalamic-pituitary-gonadal axis as a feedback system (Fig. 2). "End-organ" or "target organ" hormones regulate the release of target organ stimulating hormones. In this case, gonadotropic hormone secretion is regulated by circulating levels of androgens, the product of the "target tissue" testes. Thus, a high testosterone level inhibits gonadotropin release whereas the converse is true at low androgen levels. Although there is evidence to indicate an inverse relationship between testosterone and LH levels

(85,86) the feedback relationship controlling FSH secretion is less clear (83,87,88). There is some evidence to suggest that a metabolite of testosterone, perhaps an estrogen, rather then testosterone itself may be the regulatory factor in LH secretion (89). There is also evidence to suggest that an inhibitory factor for FSH may be secreted by the seminferous tubules (83,85-88,103). FSH and LH also appear able to regulate their own release via a "short-feedback" pathway. The pituitary-gonadal axis has been the subject of a number of reviews in animals and humans of both sexes (38,90-93).

B. GONADOTROPIN LEVELS WITH AGE

The levels of gonadotropins have been studied in all stages of development and aging (88 review). FSH and LH levels in children are low before puberty and appear to increase progressively just prior to the onset of and during puberty (62,88,94). In the sexually mature female, FSH and LH develop a cyclic pattern related to menstrual function (62,88). The sexually mature female eventually reaches an age at which ovarian failure occurs (menopause) which leads to high concentrations of LH and FSH. This rise is analagous to that seen in castrate individuals (88) when gonadotropin secretion is released from the inhibitory effects of end-organ hormones. In post-pubertal males LH and FSH levels remain relatively stable from day to day. Some diurnal variation in FSH (95) and episodic release of LH (96) has been reported. However the significance of these findings is still speculative.

In elderly males, changes in gonadotropin levels have been noted. In 1948 Pedersen-Bjergaard et al (79) documented an in-

crease in urinary gonadotropins past age sixty and reviewed earlier literature which suggests the possibility of two separate populations of elderly males; those with urinary gonadotropins in the normal range and those with elevated levels. Similar findings were reported by Albert (97). Schalch et al (98) reported elevated LH levels in male sera during the seventh and eighth decade, while Ryan and Faiman (99) have described elevated mean LH and FSH levels in males after age fifty compared to a younger group of males 20-49 years of age. The magnitude of the increase, although substantial in some individuals, was not into the castrate range. Ryan and Faiman (99) also speculate that males over fifty can be segregated into two populations based on the level of serum FSH.

If, as already suggested, total testosterone has an inhibitory or regulatory effect upon gonadotropin secretion, it is difficult to reconcile the finding of elevated gonadotropin levels in some aged males with normal total testosterone levels. However, because protein binding is elevated in elderly males, the pituitary may be recognizing a lower level of biologically active, free testosterone in spite of normal total testosterone levels. This concept is in keeping with what was discussed previously (see section on "Effects of protein-binding") where free testosterone was shown to be the biologically active fraction of the total serum testosterone concentration. Another possible reason for the elevated serum gonadotropin levels is that the aged male pituitary is no longer as responsive to feedback inhibition. However, the suppression of elevated gonadotropin levels by estrogen in old men castrated for prostatic cancer makes this possibility unlikely (10).

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SUMMARY

A binding system in plasma specific for sex steroids, particularly testosterone, has been described. The binding of testosterone by this system results in the division of the total serum concentration of testosterone into two fractions: 1) a bound fraction and 2) a free fraction.

Evidence has been reviewed which suggests that androgenic activity of testosterone corresponds better to the free testosterone fraction than to the total testosterone concentration.

Serum levels of testosterone, gonadotropins and testosterone-binding have been considered at different periods of age and development. Emphasis has been placed on the human male, specifically the aged male. Total serum testosterone levels appear to be normal or slightly lower in aged males while gonadotropin levels tend to be elevated compared to young males. Testosterone-binding is also increased with age resulting in reduced free testosterone levels. Testosterone is thought to have an inhibitory effect on gonadotropin secretion. This hypothesis is difficult to reconcile with the rising gonadotropin levels despite normal total testosterone levels in elderly males. If, as indicated, free testosterone is the biologically active fraction of the total testosterone concentration, the aged pituitary gland (or hypothalamus) may be recognizing a lower level of free testosterone which has been shown to decrease with age. This results in a diminished inhibition of gonadotropin secretion. As yet, no one has attempted to correlate total testosterone or free testosterone concentrations with gonadotropin levels in aged males nor has the

finding of increased binding in elderly males been confirmed. Because of the controversy surrounding total testosterone concentration in aged males, serum levels of testosterone also need to be restudied.

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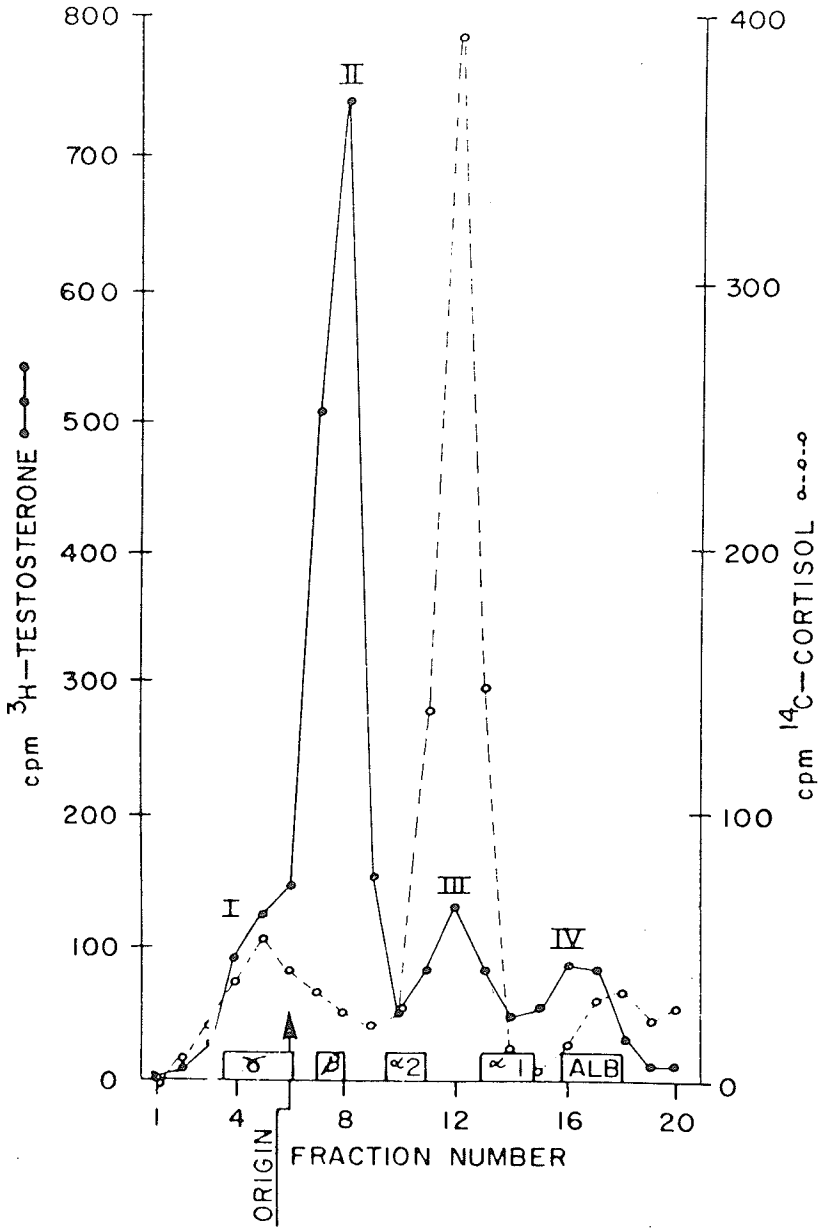

Figure 1. Simultaneous paper electrophoretogram of tracer ^{3}H-testosterone, ^{14}C-Cortisol and plasma proteins. The solid line represents the migration of ^{3}H-testosterone, and the interrupted line the migration of ^{14}C-Cortisol. The roman numerals are used to designate the peaks. Alb refers to albumin; α1, α2, β, γ refer to the globulins (18).

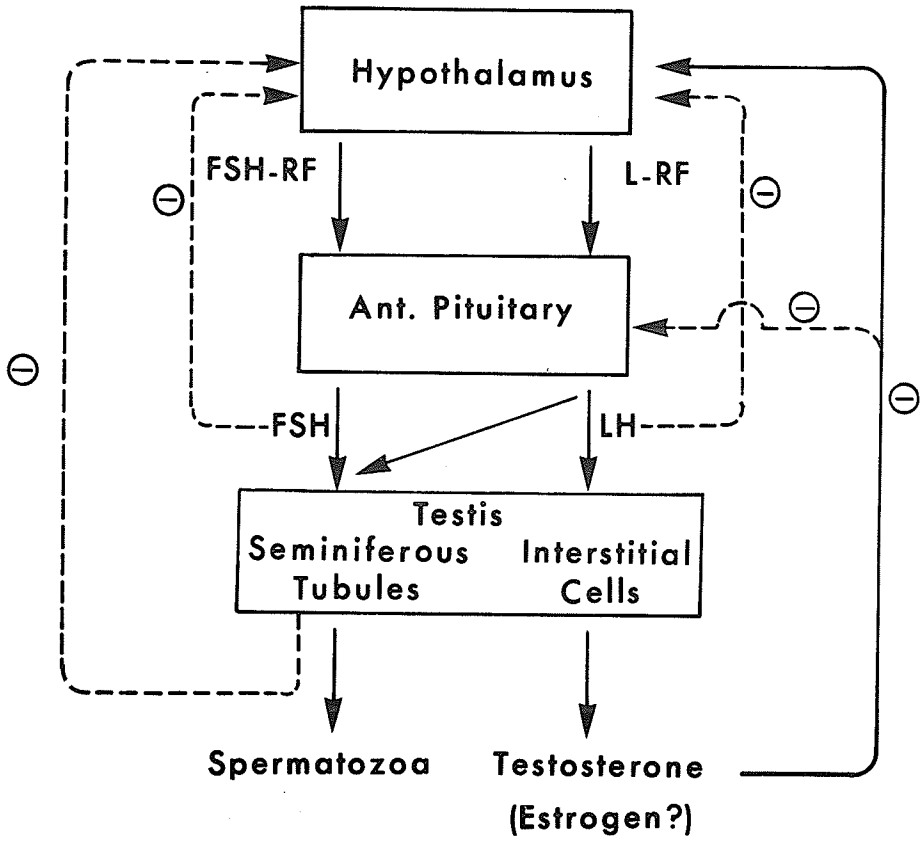

Figure 2. Feedback relationships of the hypothalamic-pituitary-testicular axis. Inhibitory pathways indicated as: ⊖
Dashed lines are, as yet, not well established pathways.

TABLE I

Intrinsic Association Constants for Binding Proteins*

Testosterone-Binding Globulin	Albumin	CBG
$4 - 16 \times 10^8$ (24)	24×10^4 (24)	—
7.5×10^8 (34)	—	$3 - 5 \times 10^6$ (34)
4.5×10^8 (29)	9×10^4 (29)	—
—	4×10^4 (33)	1.5×10^6 (33)
1.3×10^8 (52)	—	—
—	3×10^4 (3)	—
—	—	1.4×10^6 (104)

*Values in L/M

TABLE 2

PERCENTAGE OF TOTAL TESTOSTERONE BOUND BY ALBUMIN, TeBG AND CBG

	Albumin	TeBG	CBG	Free
Adult Men	30.7 (24)	65.8 (24)	1.5 (24)	1.5 (24)
	36.0 (35)	63 (35)		1.7 (35)
		58 (33)		
				2.08 (72)
				1.44 (44)
Women	11.1 (50)	85.4 (50)		0.90 (50)
		81.0 (33)	<0.2-3% (33)	0.96 (33)
				0.9 (44)
Pregnant Women		97.0 (33)	<0.2-3% (33)	0.2 (33)
		95.5 (50)		0.2 (50)
				0.27 (35)
				0.2 (44)

TABLE 3

DISPLACEMENT ACTIVITY OF VARIOUS STEROIDS FOR ^{3}H-TESTOSTERONE FROM TeBG*

Steroids with binding activity	% Activity
1. 17β-Hydroxy-5α-androstan-3-one (dihydrotestosterone)	240
2. 6α-Methyl-17β-hydroxy-5α-androstan-3-one	189
3. 5α-Androstane-3β, 17β-diol	141
4. 17β-Hydroxy-Δ^4-androsten-3-one (testosterone)	100
5. 7α-Methyl-17β-hydroxy-Δ^4-androsten-3-one	100
6. 5α-Androstane-3α, 17β-diol	98
7. Δ^5-Androstene-3α, 17β-diol	83
8. 17α-Methyl-17β-hydroxy-Δ^4-androsten-3-one (methyltestosterone)	65
9. 6β-Methyl-17β-hydroxy-5α-androstan-3-one	48
10. 6α-Methyl-17β-hydroxy-Δ^4-androsten-3-one	46
11. 9α-Fluoro-11β, 17β-dihydroxy-Δ^4-androsten-3-one	28
12. 5α-Androstan-17β-ol	22

Steroids with no significant binding activity

13. Cortisol
14. Corticosterone
15. Progesterone
16. 17α-Hydroxy-Δ^4-pregnene-3,20 dione
17. Estriol
18. Estrone
19. 3β-Hydroxy-Δ^5-androsten-17-one (dehydroisoandrosterone)
20. Androsterone
21. Etiocholanolone

*The relative activity of the various steroids was determined by comparing the displacement of ^{3}H-testosterone which results from the addition of 10-200 mμg of unlabelled test steroid to pregnancy plasma diluted 1:6 with displacement by authentic testosterone (100%). Data from Kato and Horton (16)

TABLE 4

BINDING CAPACITY OF TeBG FOR TESTOSTERONE*

MEN		WOMEN		PREGNANT WOMEN		HYPERTHYROIDISM	
$3.6 - 6.4 \times 10^{-8}$	(24)	$4.4 - 6.4 \times 10^{-8}$	(24)	$1.9 - 3.0 \times 10^{-7}$	(24)	18×10^{-8}	(34) ♀
$4.6 \pm .04$ (SE) $\times 10^{-8}$	(34)	7.4 ± 0.7 (SE) $\times 10^{-8}$	(34)			16×10^{-8}	(34) ♂
$1.7 \pm .13$ (SE) $\times 10^{-8}$	(14)†	4.9 ± 0.76 (SE) $\times 10^{-8}$	(14)†	$3.8 \pm .03$ (SE) $\times 10^{-7}$	(14)†		
$3.4 \pm .88$ (SE) $\times 10^{-8}$	(36)†	6.5 ± 2.1 (SE) $\times 10^{-8}$	(36)†				
1.44×10^{-8}	(101)	8.1×10^{-8}	(101)				

$5.2 \pm .07$ (SE) $\times 10^{-8}$	(20-50 yrs.)	
$6.5 \pm 0.8 \times 10^{-8}$	(50-70 yrs.)	}33
$8.9 \pm 1.5 \times 10^{-8}$	(70-85 yrs.)	

*Capacity is the amount of testosterone, in M/L, needed to saturate all binding sites on TeBG.

†Data derived from Corvol et al (14) and Rosenfield (36)

TABLE 5

TOTAL PLASMA TESTOSTERONE CONCENTRATIONS AND TESTOSTERONE BINDING IN CORD BLOOD

	TOTAL TESTOSTERONE CONCENTRATION (ng/100 ml)			%FREE
FETAL SEX	CORD	VEIN	ARTERY	
M+F		56±15(SD) (43)	69±17.5(SD) (43)	2.71% (50)
M	46±14(SD)	53±30(SD) (44)	-	
F		39±14(SD) (44)	-	
M		54±8.9(SE) (46)	43±5.3(SE) (46)	
F		57±9.8(SE) (46)	72±21(SE) (46)	
M		†83±12(SD) (58)	†171±17(SD) (58)	
F		56±9(SD) (58)	69±10(SD) (58)	

†significant difference

TABLE 6

TOTAL PLASMA TESTOSTERONE CONCENTRATIONS AND TESTOSTERONE BINDING IN MALES AT DIFFERENT STAGES OF PUBERTAL DEVELOPMENT

STAGE OF DEVELOPMENT	TOTAL TESTOSTERONE (ng/100 ml)		% TOTAL TESTOSTERONE UNBOUND
P1	58±8 (SE) (33)	20±9 (SD) (94)	0.86% (33)
P2	115±13 (SE) (33)	131±172 (SD) (94)	-
P3	269±36 (SE) (33)	328±111 (SD) (94)	-
P4	410±53 (SE) (33)	532±191 (SD) (94)	-
P5	608±70 (SE) (33)	605±194 (SD) (94)	1.5 (33)

Pubertal development, in reference (33), was assessed according to the criteria of Tanner based upon phallic length, testicular length and amount and distribution of pubic and axillary hair. This criteria was slightly modified in (94).

EXPERIMENTAL SECTION

INTRODUCTION

The effects of aging on testicular androgen secretion and on the hypothalamo-pituitary-gonadal axis are poorly understood in man. Data regarding serum testosterone concentrations in elderly men are contradictory. Gandy and Peterson (1), Coopage and Cooner (2), and Kent and Acone (3) were unable to demonstrate a significant difference in mean serum testosterone concentrations between young and old men up to the age of 80. In contrast Vermeulen et al (4,5) reported significantly lower mean serum testosterone levels in men after the 7th decade while Persky et al (6) reported lower mean levels after the 3rd decade. Urinary excretion studies of testosterone glucuronide have shown a trend towards lower values in men after the age of forty (7-11). Several authors reported a greater variability in values in aged men when compared to those observed in younger subjects (2,4,5).

Serum and urinary gonadotropins have been reported to be elevated in elderly men (8,12-14). Ryan and Faiman (14) speculated that elderly males fall into two groups: 1) those with normal serum gonadotropin levels and 2) those with elevated gonadotropin levels. A similar conclusion was reached based upon urinary gonadotropin estimations (8,12).

The finding of elevated gonadotropin levels despite what may be normal total serum testosterone concentrations appears to be unexplained by current concepts of gonadotropin-testosterone feedback interrelations (15). Evidence is accumulating however, which suggests that the biological activity of testosterone resides in the fraction of testosterone unbound from serum proteins (5,16-19). It follows that

the unbound fraction rather than the total testosterone concentration may be the feedback determinant of gonadotropin regulation. Thus, the finding of reduced unbound testosterone fractions in aged males by Vermeulen et al (4) may explain the finding of elevated serum gonadotropin levels in the presence of normal total testosterone concentrations.

The aims of the present study were to re-examine the levels and interrelationships among total testosterone, free testosterone and gonadotropin levels with aging.

Methods and Materials

Subjects: One hundred and forty-six male subjects, aged 20-93 years were studied. Eighty-eight, aged 65-93 years, were ambulatory outpatients at Deer Lodge Veterans Hospital. All were in good health on the basis of a physical examination and routine laboratory studies (hemoglobin, urinalysis, BUN) and none were known to have any endocrine disorders. Fifty-eight were healthy hospital personnel and Red Cross Blood Donors between 20 and 64 years of age. In order to validate the testosterone-binding assay to be described, eight children, nine normally cycling women, six women taking a combined-type oral contraceptive and thirteen pregnant women (3rd trimester) were also studied.

Blood Samples: Twenty ml samples of blood were taken by venepuncture between 8 and 11 a.m., allowed to clot at room temperature, and sera frozen at -20C until analyzed for FSH, LH, testosterone and testosterone-binding.

Gonadotropins: Serum FSH and LH levels were measured in duplicate by the double-antibody radioimmunoassay methods of Faiman and Ryan (20,21). Values are expressed in terms of the crude human pituitary standard LER-907. The coefficients of variance for duplicate determinations

were ±6% for LH and ±5% for FSH.

Serum Testosterone: Testosterone was measured by a radioimmunoassay technique which utilized an antiserum raised in rabbits against 4-androsten-17β-ol-3-one-O-carboxyoxime (Steraloids), conjugated at the C-3 position with bovine serum albumin (Schwarz/Mann) by the method of Gocke et al (22). Duplicate standards (0,50,100,150,200,300 and 400 pg) and serum samples (0.5 ml for men and 2.0 ml for women and children) were brought to a volume of 2 ml with distilled water and extracted with 12 ml methylene chloride. The extracts were washed successively with 2 ml 1.0 M pH 10.3 bicarbonate buffer, 1.5 ml of 1.0 M $NaHCO_3$ and 2 ml water; 4 ml of aliquots of each extract were dried under N_2 at 37 C. To each tube 0.1 ml of dilute antiserum (diluted 1:300 in 1.0 M phosphate-buffered saline (pH 7.4) containing 0.1% gelatin) was added; the tubes were shaken and incubated for 30 minutes at 37 C. Testosterone-1, 2-H^3 (New England Nuclear, S.A. 45 Ci/mM) (6500 cpm in 0.1 ml buffered saline) were then added, the tubes were shaken and the incubation continued at 4 C for 30 minutes. To each tube was added 0.1 ml 0.5% gelatin in phosphate-buffered saline plus 1.0 ml dextran-coated charcoal (0.1 gm charcoal (Norit A) and 0.01 gm dextran-80 (Pharmacia)/100 ml distilled water); the tubes were shaken, incubated for 3 minutes at 4 C and centrifuged for 2 minutes at 2500 rpm. A 0.5 ml aliquot of supernatant was taken for liquid scintillation counting and a standard curve was constructed. The sensitivity of this method was 2 ng/100 ml when a 2 ml sample was assayed. No correction for blanks or recovery, normally about 80%, was made as the standards were treated in the same fashion as the samples. The coefficient of variation between duplicate determinations was ±7%. Table 1 demonstrates the specificity of this

method.

Percent Free Testosterone: An index of testosterone-binding was derived using the method of Rosenfield (23) with the following modifications: Testosterone-1,2-H^3 (as above) was used without further purification. To each assay tube was added 1.0 ml of a phosphate buffer containing 44,000 dpm (0.1 ng) of testosterone-1,2-H^3. One ml of a 0.1% charcoal, 0.01% dextran-80 (Pharmacia) suspension was then added in order to separate bound from free testosterone. The unbound tracer is completely removed (<5%) in the absence of binding-proteins. The cpm in 0.25 ml of supernatant, rather than 0.33 ml, was used to calculate percent testosterone bound. Scintillation counting was performed in a Unilux II scintillation counter (33% efficiency for H^3) using a modified Bray's scintillation fluid.

The intra-assay coefficient of variation between duplicate determinations was ±2.5%. The serum of two adult men and one woman bound 54.5±1.2% (SD) (n=11), 49.3±1.4% (n=9) and 75.4±1.9% (n=13) of the labelled testosterone respectively upon repetitive analysis; the inter assay coefficients of variation were thus ±2.2%, ±3.0% and ±2.6% respectively. Because of the increased precision compared to Rosenfield's report (23) the control samples used to correct for inter assay variation were not found to be necessary.

Testosterone-Binding Index: The testosterone-binding index was calculated by multiplying the percent free testosterone value by the total serum testosterone concentration value for each subject. This yields an index which quantitates the unbound testosterone concentration.

Statistical Analysis: A computer program for multiple-discriminant analysis (25,26) was used to determine the age which best separates the 146 men studied into two groups, a "young" and an "aged" group, based on

the five variables; total testosterone, percent free testosterone, testosterone-binding index, FSH and LH. A computer program was then used to determine the linear and log-linear correlations (26) among the six variables (age, FSH, LH, total testosterone, percent free testosterone and testosterone-binding index) for the overall group of 146 men and for the "young" and "aged" groups respectively.

Results

Total Testosterone: Serum testosterone levels vs age are shown in Figure 1. There was a pronounced overall decline in mean testosterone levels, with a wide range in values, in subjects over 70 years of age. However, in the great majority of men, serum testosterone levels were maintained within the limits for young men (230-1100 ng/100 ml) up to and including the 9th decade. Although the majority of subjects over 75 years of age had levels indistinguishable from those seen in young men, 18 of 70 had testosterone levels below 230 ng/100 ml, the lower limit for young men; two with levels as low as those seen in castrate subjects (<50 ng/100 ml).

Percent Free Testosterone: Figure 2 shows the percent free testosterone in various states. The free testosterone levels were in descending order: young men>old men>women>children>women on "pill">pregnant women. Old men had a mean percent free testosterone level intermediate between young men and women and there was good agreement between the values obtained in the present study compared to those of Rosenfield (23). Figure 3 shows the effect of age on percent free testosterone. Levels declined after about age 40 and were considerably reduced in men>70 years of age (20-45%) compared to men less than 40 years (43-60%).

Testosterone-Binding Index: The testosterone-binding index (Figure 4) appeared to remain constant, with a wide range of values, until the

5th decade at which point an overall decline occurred. In contrast to what was seen for total testosterone levels, far more subjects had levels less than the lower limit for young men; 2 of 30 subjects aged 50-70 years and 37 of 83 subjects over 70 years of age had levels below 100, the apparent lower limit for young men.

Gonadotropins: FSH and LH concentrations are plotted as a function of age in Figures 5 and 6 respectively. Subjects below age 45 (with one exception) had FSH values between 8-30 μg/100 ml and LH values between 2.5-7.5 μg/100 ml. There was a tendency towards increasing values between ages 45-70; 14 of 35 subjects had FSH values above 30 μg/100 ml and 15 of 35 had LH values above 7.5 μg/100 ml. After age 70 there was a marked increase in the range of FSH and LH values, 47 of 86 men had FSH values above 30 μg/100 ml while 54 of 86 had LH levels above 7.5 μg/100 ml. Of the men with elevated gonadotropin levels, 16 had FSH and 22 had LH values in the post menopausal range (FSH>100 μg/100 ml; LH>12 μg/100 ml).

INTERRELATIONSHIPS: Since changes were observed in total testosterone, percent-free testosterone, testosterone-binding index and gonadotropin levels with age, it was decided to determine which age best separated the subjects into a "young" and an "old" group based on these 5 variables. Accordingly, a multiple-disciminant analysis was performed and the determined age found to be 45 years. There were 30 subjects in the young group and 116 in the old. Comparison of the weighted contribution (25) of each variable to the discriminant function showed that the percent free testosterone was the major contributor.

All variables were significantly ($p<0.001$) correlated with age both overall (Table 2) and also within the old group. None were

correlated with age in the group of men less than 45 years of age. Logarithmic transformation of the variables improved the overall correlations of total testosterone, FSH and LH with age (Table 2).

FSH and LH, each as a function of total testosterone, percent-free testosterone and the testosterone-binding index, are shown in Figures 7-12. In all cases a curvi-linear relationship was observed. The overall patterns were similar for both FSH and LH. This is not surprising since FSH and LH were significantly correlated with each other overall ($r=0.758$, $p<0.001$) and within the young ($r=0.515$) and old ($r=0.744$) groups. As the patterns relating FSH and LH to the testosterone variables appeared to be non-linear, logarithmic transformation of all 5 variables was done in order to better linearize the data.

FSH and LH were each significantly inversely correlated with total testosterone, percent-free testosterone and the testosterone-binding index overall (Table 3). The inverse correlation remained highly significant in men>45 (Table 5) but not in the young group. Logarithmic conversions improved these correlations in all but the young group (Table 3 and 5). Transformation of the data did not result in significant correlations within the young group.

FSH correlated best with percent-free testosterone, both overall and within the old group, whereas LH correlated approximately equally well with the percent-free testosterone and the testosterone-binding index (Tables 3 and 5). Of note is the finding that the percent-free testosterone and the index of binding, both parameters of the free testosterone concentration, correlated better with FSH and LH than did the total testosterone concentration.

It is evident from Figures 8 and 11 that the overlap between young and old groups is greatly reduced when percent-free testosterone rather than total testosterone levels are considered. This is in keeping with the observation that percent-free testosterone was the best disciminator between young and old men.

DISCUSSION

From the present study it is evident that total serum testosterone levels decline overall after age 70, yet remain within the limits for young men in the majority of subjects. This is in general agreement with Vermeulen et al (4) who noted a decline in mean testosterone levels after age 60. Because of the wide range of values and the small number of elderly subjects studied it is not surprising that Gandy and Peterson (1), Coppage and Cooner (2) and Kent and Acone (3) were unable to observe a significant change in mean testosterone levels with age.

The decline in the free testosterone level after age 40 in our study confirms the findings of Vermeulen et al (4,5) who observed a similar decrease in the free plasma testosterone concentration with age. The wide range of values for the testosterone-binding index and the free plasma testosterone concentration reflects the wide range of testosterone values seen in both studies. Both the percent-free testosterone and testosterone-binding index show an earlier and more pronounced decline with age than do total testosterone levels. Metabolic studies (16-19,27,28) suggest that it is the free fraction which is biologically active, so that while overall total testosterone levels are within normal limits, the biologically effective testosterone level is reduced in elderly men.

The blood production rate (BPR) of testosterone has been shown to be reduced in men past middle age (3,6,29). The decreased BPR no doubt accounts for the low testosterone levels seen in some subjects over 70 but does not appear compatible with the testosterone levels seen in the majority of subjects. However, Kent and Acone (3), Vermeulen et al (4) and Persky et al (6) have shown that the metabolic clearance rate (MCR) of testosterone is also reduced in male senescence. As serum testosterone levels are a direct function of the BPR and an inverse function of the MCR, a decreased MCR may offset a decreased BPR so that normal testosterone levels result. This probably explains the normal levels (compared to young adult levels) seen in the majority of elderly subjects.

The reduced MCR appears to be a consequence of the decreased free testosterone levels observed in elderly men since Vermeulen et al (4) have demonstrated a positive correlation between free testosterone levels and the MCR. The regulation of levels of serum proteins binding testosterone is still speculative. Estrogen has been shown to increase binding protein levels (30-32) while testosterone administration in man has been shown to decrease binding protein levels (27,31,33). Possibly a very subtle decrease in total testosterone levels results in increased binding protein levels. Whether these or other unknown etiological factors are responsible for the increased binding seen in elderly men is unclear.

The data show that FSH and LH levels rise after age 40 so that by age 80 normal-to-elevated levels are seen, some in the same range as those seen in post-menopausal women. Our data are in agreement with the findings of Schalch et al (13) who observed elevated LH levels

in men during the 7th and 8th decade. Ryan and Faiman (14) also observed elevated FSH and LH levels in men>50 years of age. The parallelism between FSH and LH levels, overall and within the young and old groups, is in good agreement with previous literature (34). Although there is a wide scatter in FSH and LH values, there is no evidence of two separate populations within the old group as suggested by others (13,14) as values appear continuous, rather than segragated into two distinct populations.

Rising gonadotropin levels are inversely correlated with decreasing total testosterone levels, but especially with the percent-free testosterone and testosterone-binding index levels. This is in keeping with the concept of diminished testosterone production in the elderly male with a compensatory increase in FSH and LH levels (15).

Reduced testosterone production by the testis may not be the sole cause for the elevated FSH and LH levels observed in elderly men. There is evidence to suggest that a hormone secreted by the seminiferous tubules is responsible for regulating FSH secretion (34). Histologically there are no marked changes in testicular structure in the aged testis except for some thinning of the germinal epithelium and a slight reduction in the number of Leydig cells. The tubules are of adult proportions but may be slightly distended and the interstitium may be less fibrous (35,36). Despite this apparent lack of significant structural change with age, functional impairment of the seminiferous tubules in FSH feedback regulation remains a possibility.

Recent evidence suggests that a metabolite of testosterone, possibly an estrogen, rather than testosterone itself, may be the feedback regulator of LH secretion (37). Formation of this metabolite(s) may be a function of the free testosterone fraction which may account for the highly significant correlation between gonadotropin levels and the free testosterone fraction.

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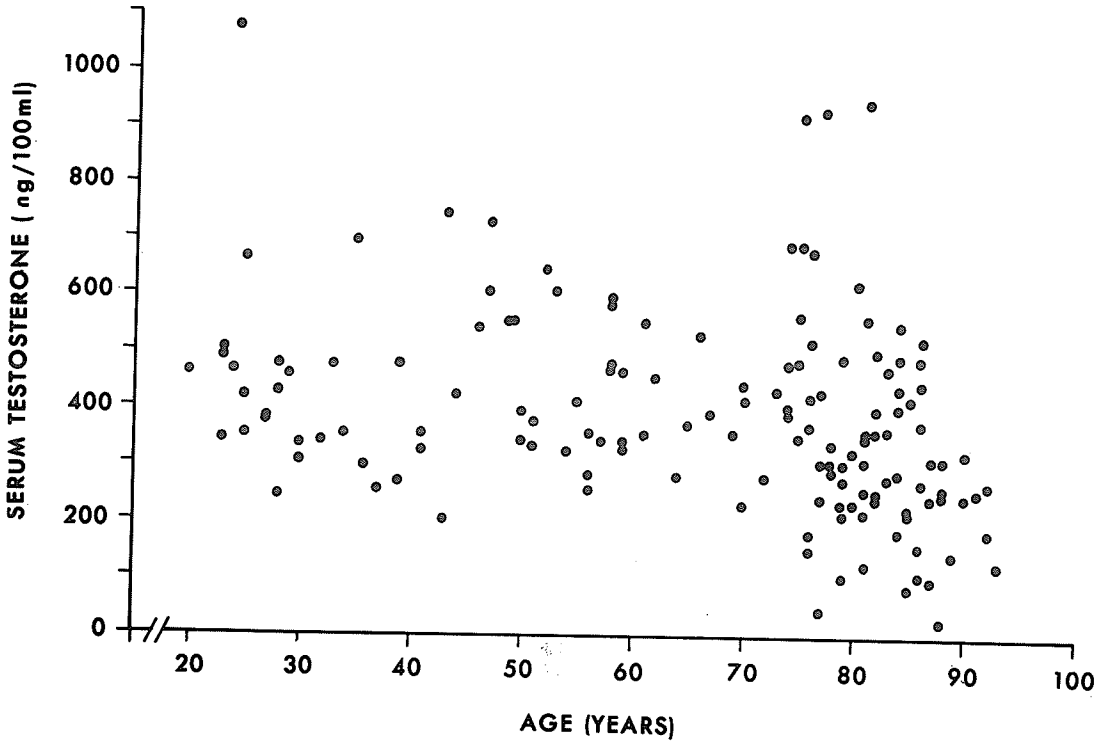

FIGURE 1. Serum testosterone concentrations in men at different ages.

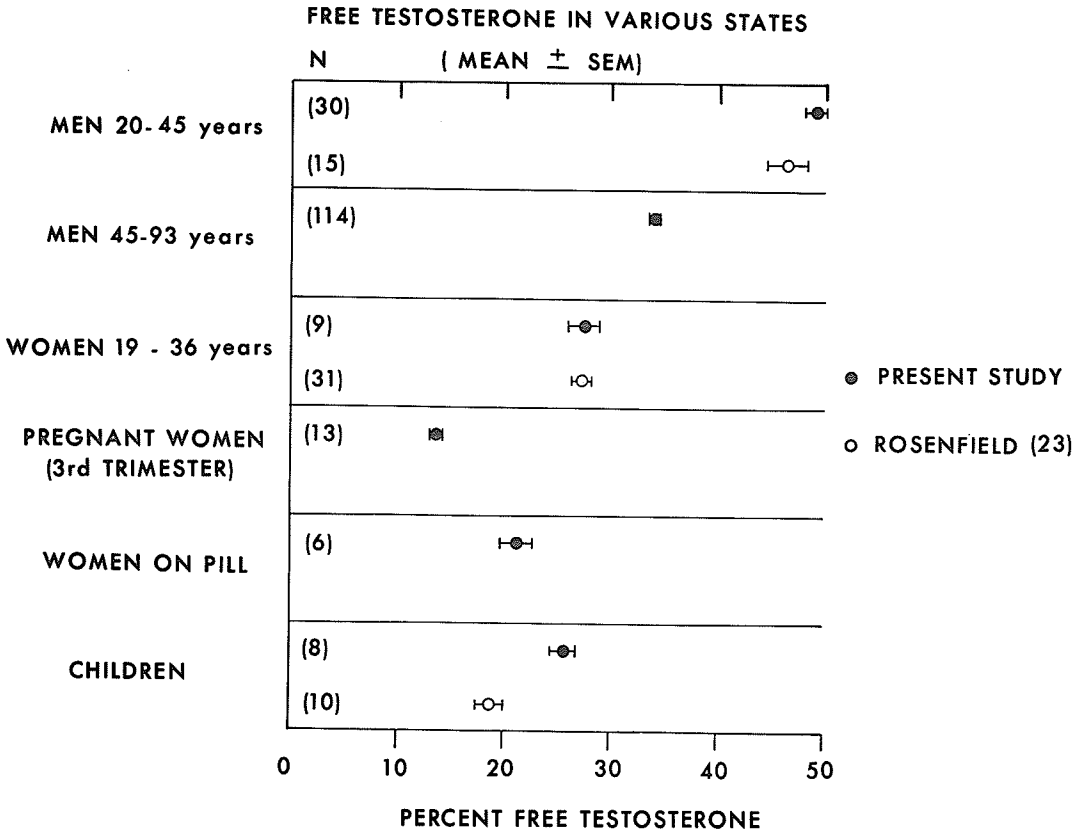

FIGURE 2. Percent free testosterone levels (mean ± SEM) in subjects with different endocrine status. Results of Rosenfield's study are represented by open circles while results of the present study are presented by closed circles.

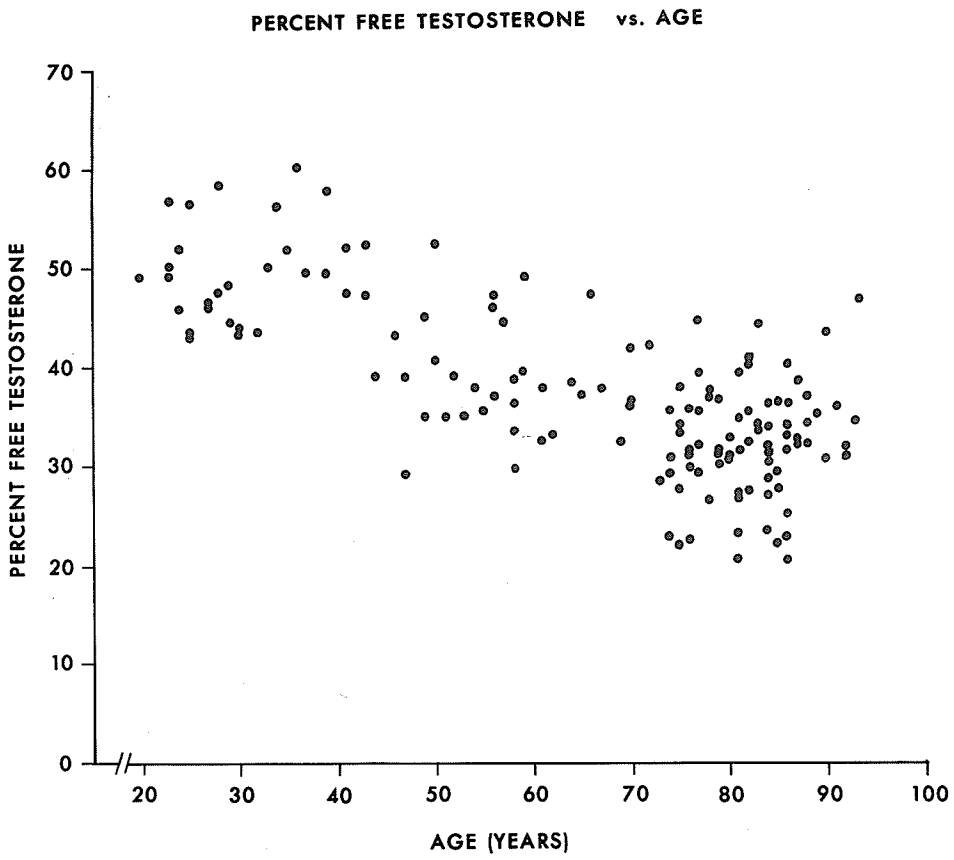

FIGURE 3. Percent free testosterone levels in men at different ages.

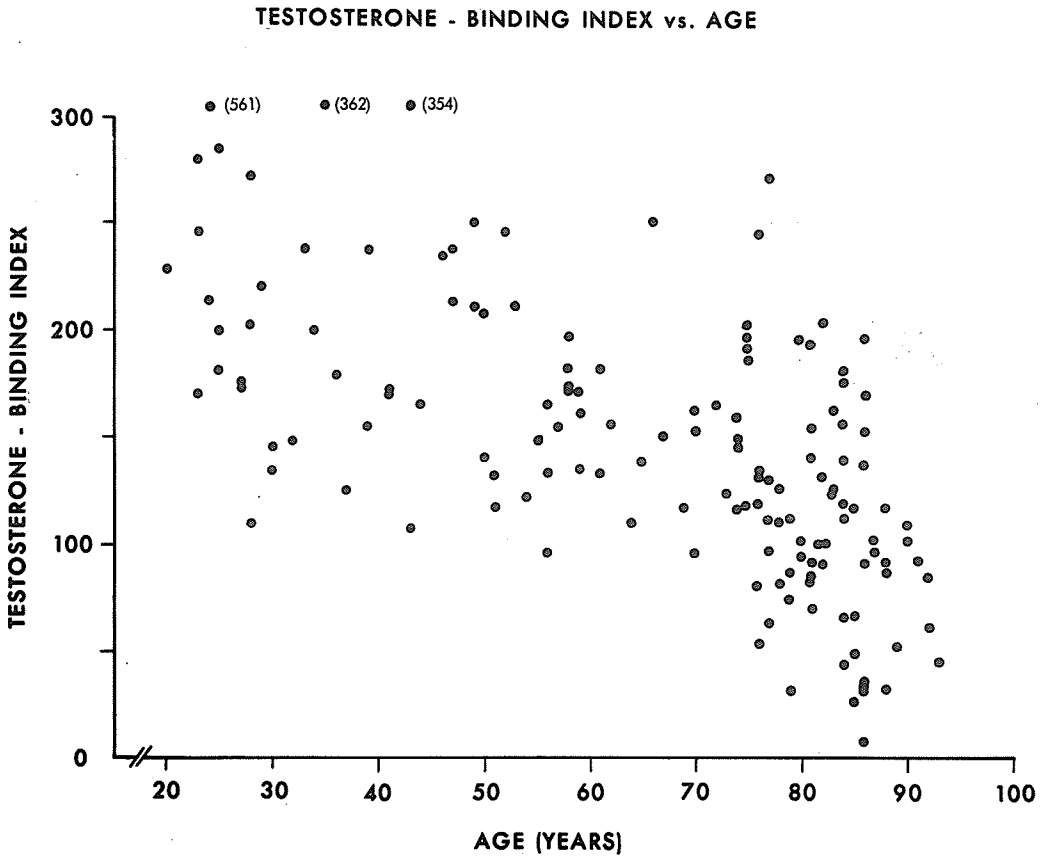

FIGURE 4. Testosterone-binding index levels in men at different ages (see text for derivation of index).

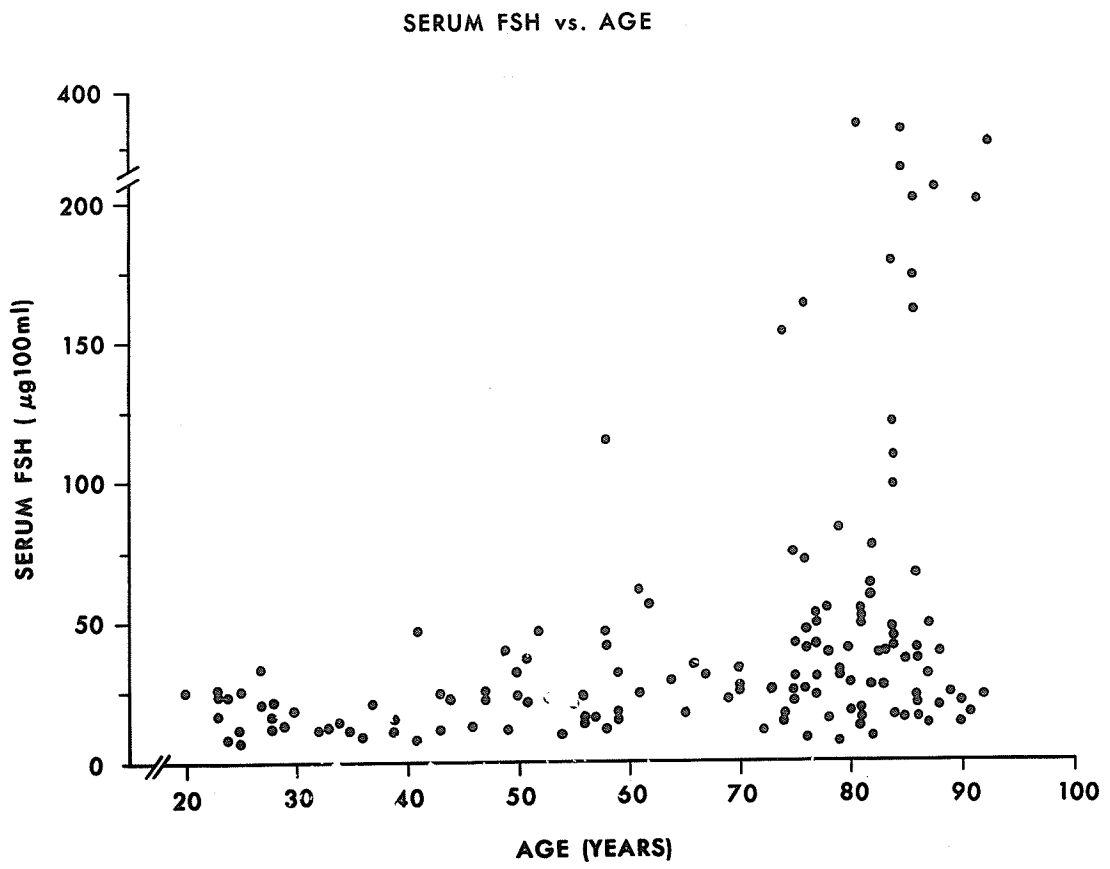

FIGURE 5. Serum follicle stimulating hormone concentrations in men at different ages.

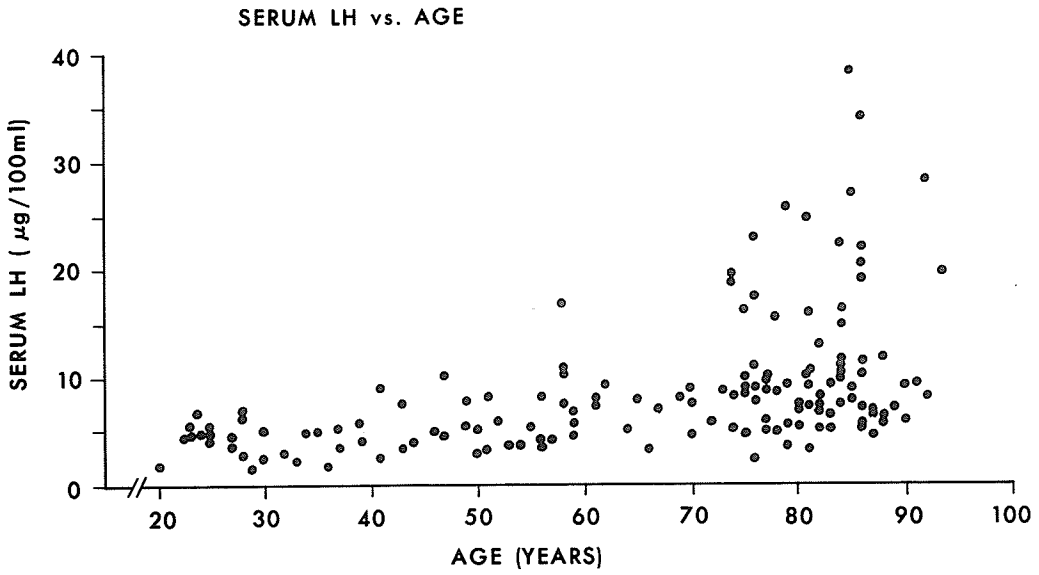

FIGURE 6. Serum luteinizing hormone concentrations in men at different ages.

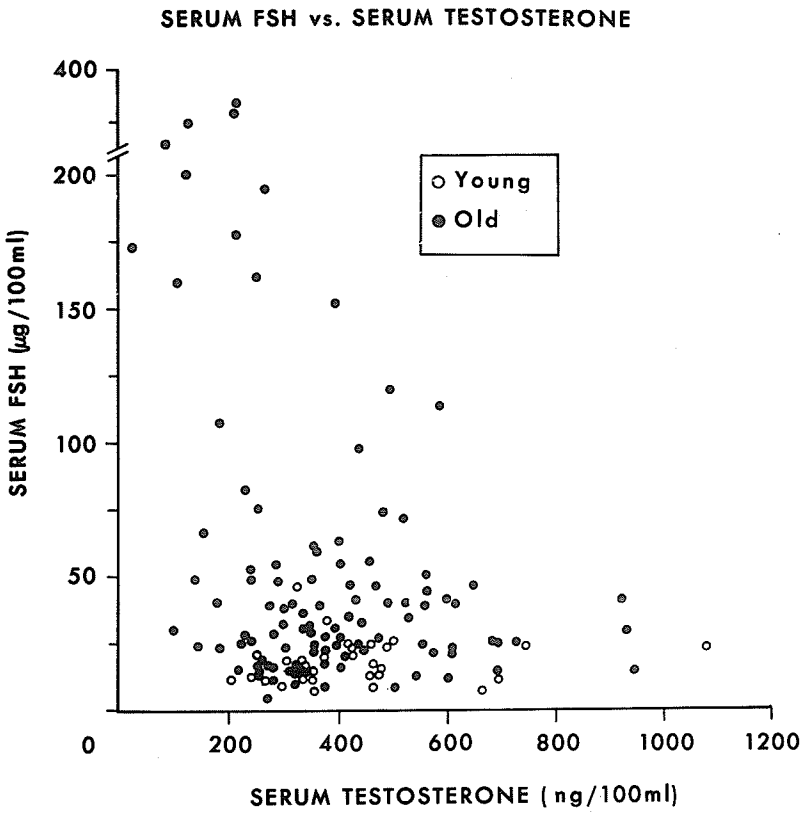

FIGURE 7. Serum follicle stimulating hormone concentrations as a function of serum testosterone concentrations. Open circles represent men 20-45 years of age, closed circles represent men >45 years of age.

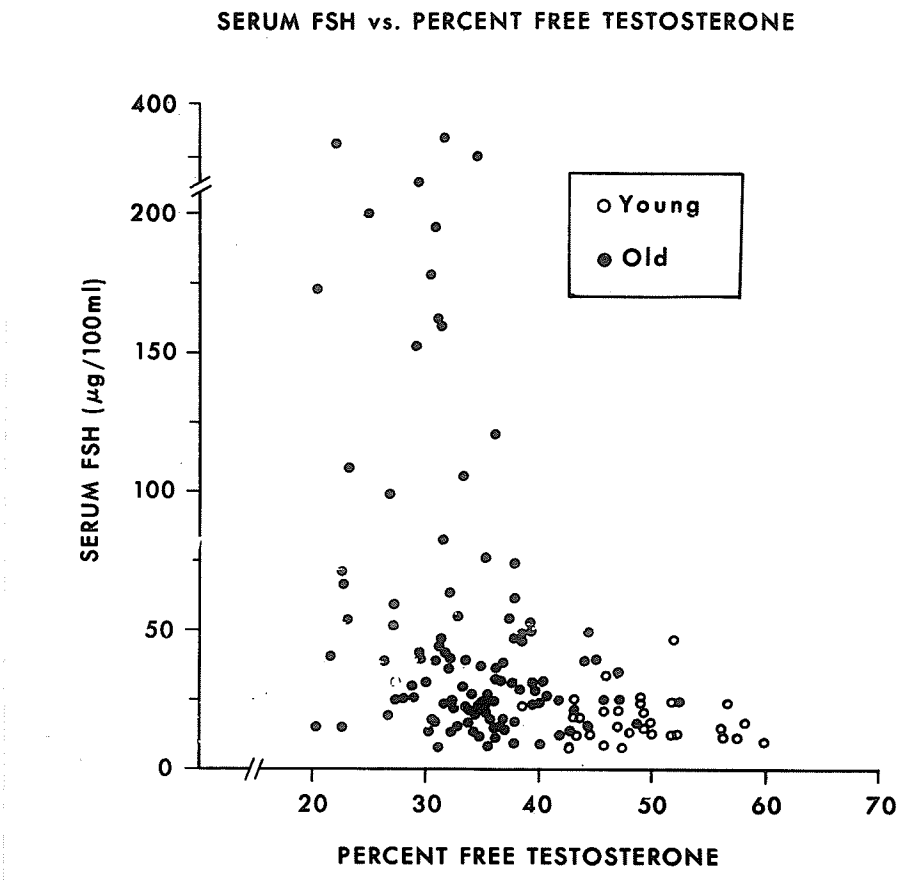

FIGURE 8. Serum follicle stimulating hormone concentrations as a function of the percent free testosterone. Open circles represent men 20-45 years of age, closed circles represent men >45 years.

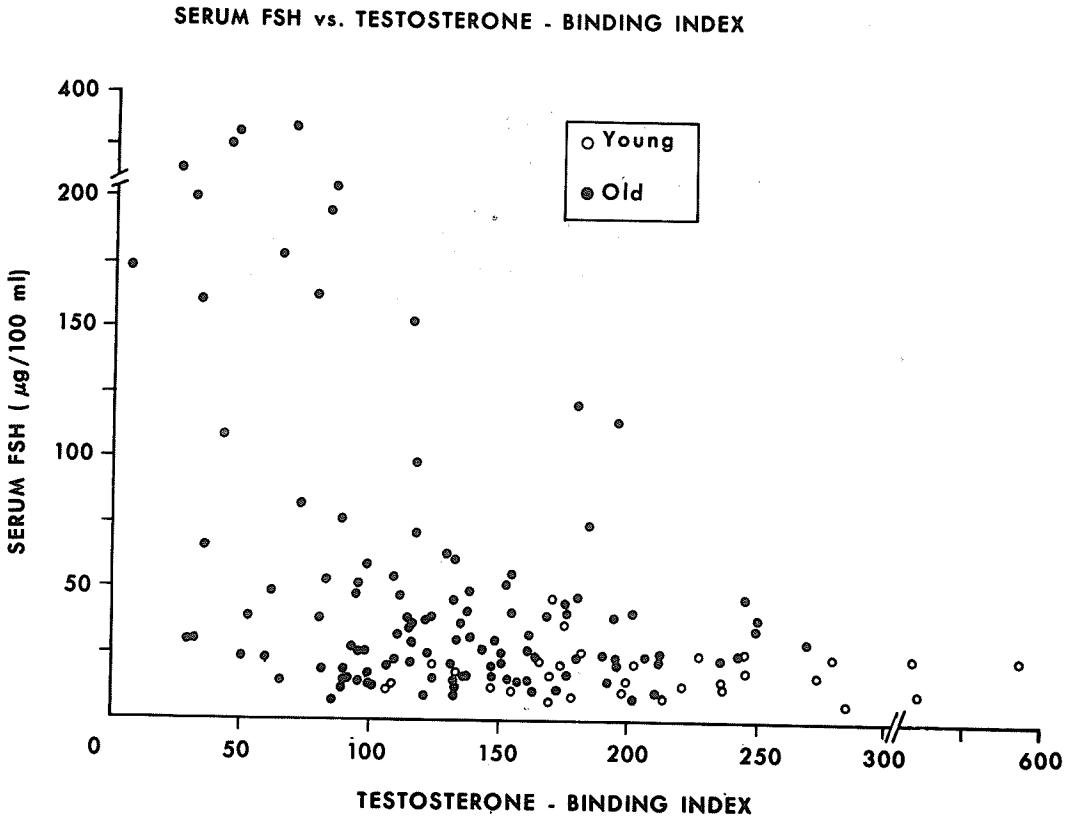

FIGURE 9. Serum follicle stimulating hormone concentrations as a function of the testosterone-binding index. Open circles represent men 20-45 years of age, closed circles represent men >45 years.

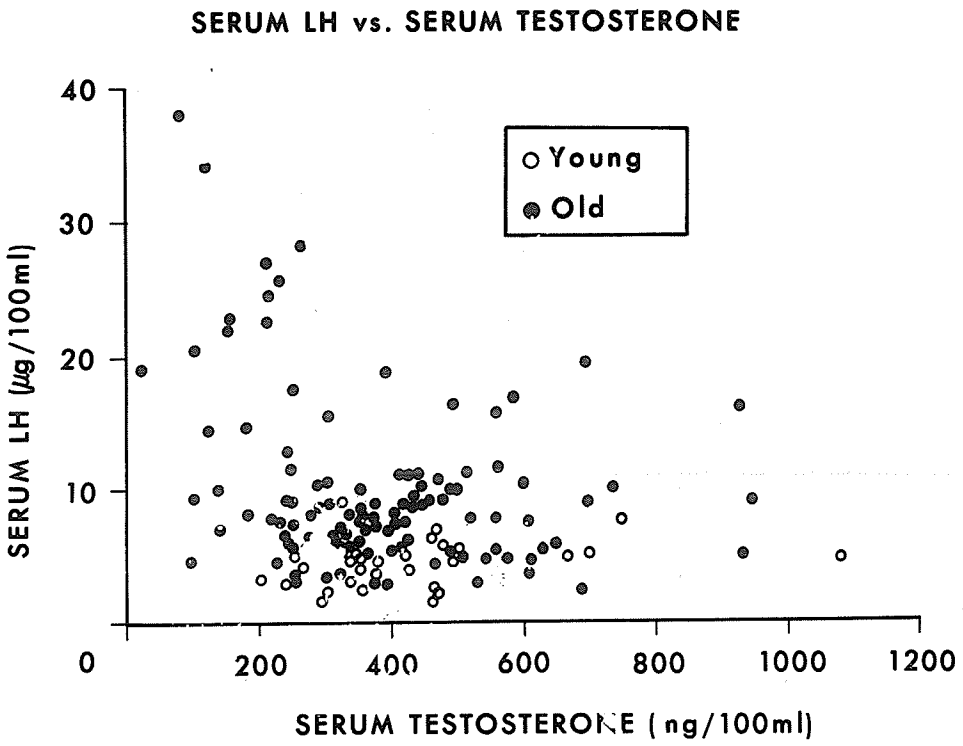

FIGURE 10. Serum luteinizing hormone concentrations as a function of serum testosterone concentrations. Open circles represent men 20-45 years of age, closed circles represent men >45 years.

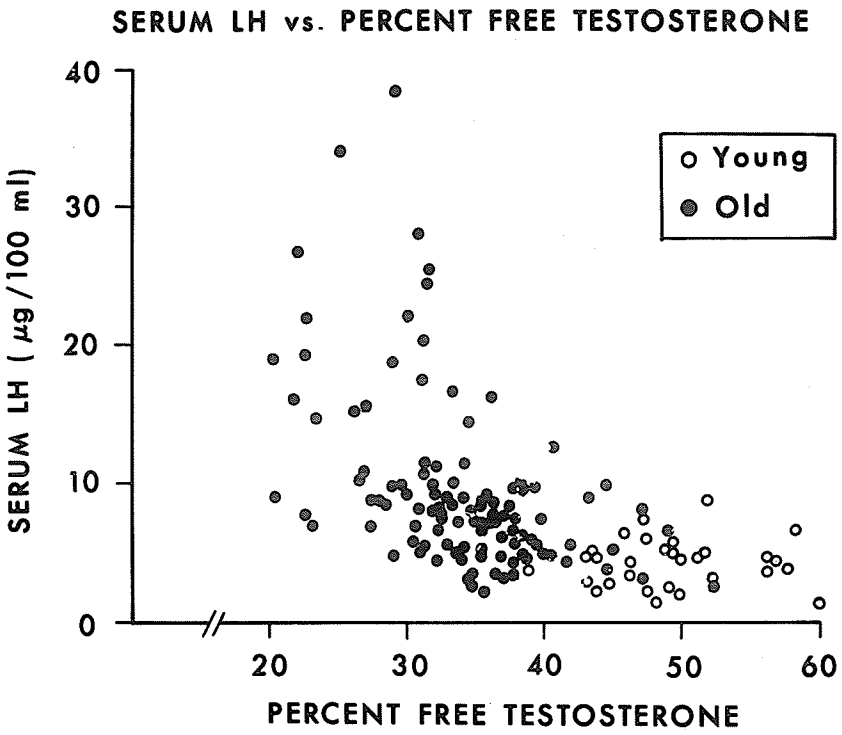

FIGURE 11. Serum luteinizing hormone concentrations as a function of the percent free testosterone. Open circles represent men 20-45 years of age, closed circles represent men >45 years.

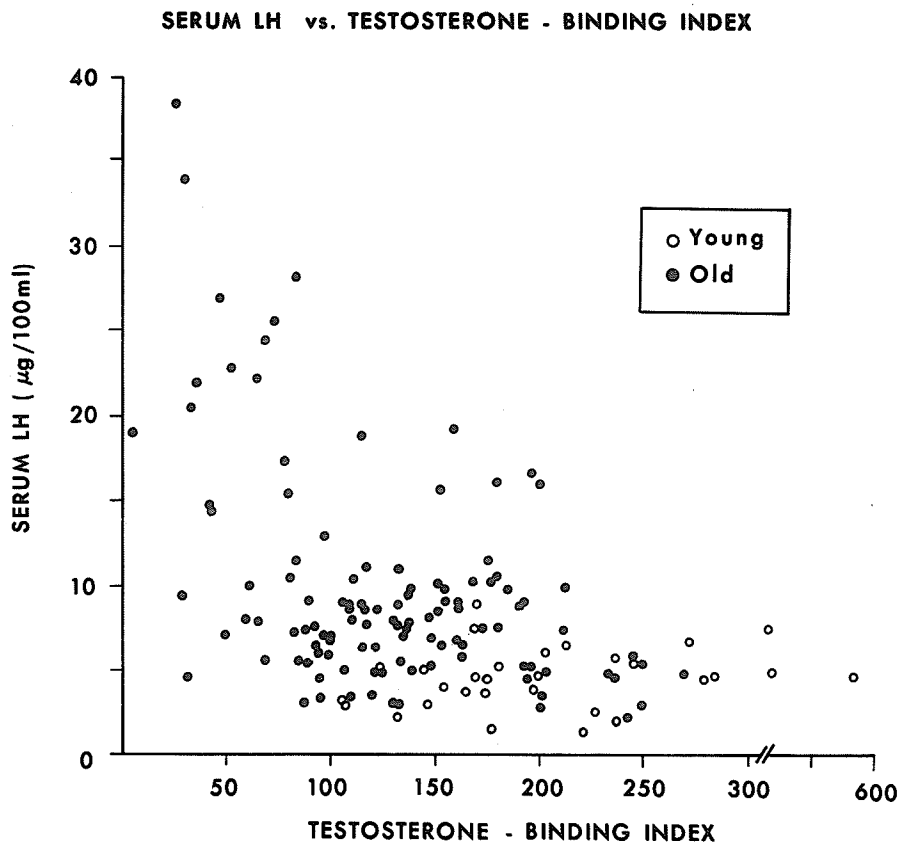

FIGURE 12. Serum luteinizing hormone concentrations as a function of the testosterone-binding index. Open circles represent men 20-45 years of age, closed circles represent men >45 years.

TABLE 1

Specificity of Serum Testosterone Assay*

Steroid	Percentage Interference
1. Dihydrotestosterone	50
2. 5α-Androstan-3α, 17β-diol	2.5
3. 1,4-Androstadien-17β-ol-3-one	30
4. Hydrocortisone	<1
5. Corticosterone	<1
6. 4-Pregnen-20α-ol-3-one	<1
7. 4-Pregnen-17α-21,-diol-3,20-dione	<1
8. Epitestosterone	<1
9. 5α-Androstan-3β, 17β-diol	1.5
10. 4-Androsten-3β, 17β-diol	5.4
11. Δ^4-Androstenedione	1.0
12. Estrone	<1
13. Dehydroepiandrosterone	<1
14. Pregnanediol	<1
15. Pregnanetriol	<1
16. Estriol	<1
17. Etiocholanolone	<1
18. Androsterone	<1
19. 5α-Androstan-3β-ol-17 -one	<1
20. 17α, 21-hydroxy-Δ^4-pregnene-3,11,20-trione	<1
21. 21-hydroxy-Δ^4-pregnene-3, 20-dione	<1
22. 11β-hydroxy-Δ^4-pregnene-3, 20-dione	<1
23. 17α-hydroxy-Δ^4-pregnene-3, 20-dione	1.54
24. Progesterone	<1
25. Estradiol	<1
Synthetic Drugs	
1. Stanozolol (Winstrol-Winthrop)	<1
2. Medroxyprogesterone actetate (Provera-Upjohn)	<1
3. Norethindrone-Mestranol (Ortho-Novum-Ortho)	<1
4. Ethisterone (Lutocylol-Ciba)	1.5
5. Norethandrolone (Nilevar-Searle)	<1
6. Oxymetholone (Adroyd-P.D. and Co.)	<1
7. Dydrogesterone (Duphaston-Will)	<1
8. Fluoxymesterone (Ultandren-Ciba)	<1
9. Norethynodrel-Mestranol (Enovid-Searle)	1.2
10. Ethinyl Estradiol	<1
11. Methytestosterone (Metandren-Ciba)	1.8
12. Clomiphene citrate (Clomid-Merrell)	<1

*Specificity of the method was assessed by assaying 5 ng of various steroids through the entire method. The percent interference was calculated by comparing the amount of steroid measured with the actual amount of steroid in the sample.

TABLE 2

OVERALL CORRELATIONS (r values) OF TOTAL TESTOSTERONE, PERCENT FREE TESTOSTERONE, INDEX OF BINDING, FOLLICLE STIMULATING HORMONE AND LUTEINIZING HORMONE VERSUS AGE (n=146)

Variable	Linear Correlation	Logarithmic Linear Correlation
Testosterone	-0.293*	-0.339
% Free Testosterone	-0.733	-0.698
Index of Binding	-0.614	-0.571
FSH	0.362	0.456
LH	0.460	0.566

*$p<0.01$

$p<0.001$ for all other values

TABLE 3

OVERALL LINEAR AND LOG-LINEAR CORRELATIONS (r values) OF FSH AND LH VERSUS TOTAL TESTOSTERONE, PERCENT FREE TESTOSTERONE AND THE INDEX OF BINDING (n=146)

	Testosterone	% Free Testosterone	Index of Binding	Log Testosterone	Log % Free Testosterone	Log Index of Binding
FSH	-0.321	-0.376	-0.396	-0.425	-0.390	-0.522
Log FSH	-0.254	-0.470	-0.392	-0.353	-0.474	-0.494
LH	-0.258	-0.542	-0.438	-0.385	-0.566	-0.558
Log LH	-0.187	-0.623	-0.430	-0.296	-0.634	-0.509

TABLE 4

LINEAR AND LOG-LINEAR CORRELATIONS (r values) WITHIN THE YOUNG GROUP (n = 30)

	Testosterone	% Free Testosterone	Index of Binding	Log Testosterone	Log % Free Testosterone	Log Index of Binding
FSH	0.091	-0.095	0.080	0.108	-0.084	0.080
Log FSH	0.109	-0.106	0.104	0.125	-0.096	0.094
LH	0.258	0.052	0.259	0.286	0.062	0.297*
Log LH	0.264	0.008	0.257	0.281	0.017	0.279

*$0.05<p<0.1$; all remainder $p>0.1$

TABLE 5

LINEAR AND LOG-LINEAR CORRELATIONS (r values) WITHIN THE OLD GROUP (n = 116)

	Testosterone	% Free Testosterone	Index of Binding	Log Testosterone	Log % Free Testosterone	Log Index of Binding
FSH	-0.343	-0.322	-0.429	-0.431	-0.327	-0.504
Log FSH	-0.278	-0.340	-0.377	-0.365	-0.344	-0.451
LH	-0.280	-0.498	-0.463	-0.395	-0.507	-0.532
Log LH	-0.230	-0.546	-0.457	-0.331	-0.548	-0.486